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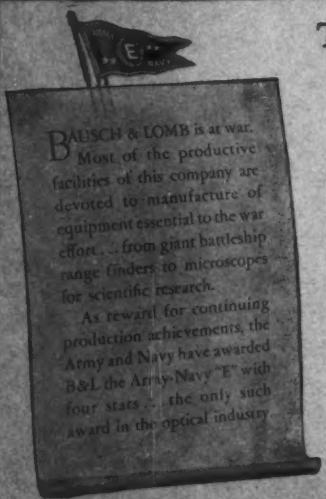
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CALCIFICATION OF THE MEDIA OF THE HUMAN AORTA AND ITS RELATION TO INTIMAL ARTERIOSCLEROSIS, AGEING AND DISEASE *

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In recent years the emphasis in the etiology of atheromatosis and arteriosclerosis has been placed on the intimal changes. This has been based mainly on the fact that numerous experiments in animals, particularly in the rabbit, have demonstrated the production of atheromatous lesions following the administration of large amounts of lipoid substances such as cholesterol. However, there is also evidence that other factors play an important rôle in the genesis of arteriosclerosis and that the lipoids may be secondarily deposited in the intima. It has not been possible to show, except in rare instances, that there exists in human beings an elevated lipoid content of the serum which might account for the presence of atheromatous lesions in the intima of the aorta. The experiments of Anitschkow [1] and also of Harrison [2] have demonstrated that the presence of medial defects in the rabbit's aorta will influence the deposition of intimal lipoids following cholesterol feeding. There are also certain histological differences between the character of the intimal lesions in experimental animals and in man, although these differences may be considered of relatively minor importance.

The association between the elastic properties of arteries and the development of the intimal lesions of arteriosclerosis has been a point of interest for many years. It appears to have been first stressed by Polotebnow,[3] in 1868, who observed that a sclerotic artery could be stretched only one-fourth as much as a normal one. Kaufmann [4] found that the size of the aorta depended on age and body build, the enlargement of the aorta with age being due to a loss of elasticity. More recently the experiments of Wilens [5] showed that with advancing age there is a progressive loss of elasticity which varies in degree in differ-

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ent areas but proceeds at a constant rate for a given area and affects all persons to approximately the same degree. He has further observed that the development of intimal plaques is not directly related to this loss of elasticity, for a greatly thickened intima can restrict the elasticity of the media only to a relatively slight degree. Loss of elasticity of the media proceeds as rapidly in those subjects who develop slight intimal atheromas as in those who exhibit profound changes. The earliest and largest intimal accumulations of lipoids appear in those areas which are subject to the earliest and most marked loss of elasticity.

The theory that loss of elasticity and weakening of the vessel wall are the primary factors that precipitate all subsequent intimal lesions is an old one. It was propounded by Thoma and Kaefer,[6] in 1889, and has been supported by Faber,[7] Klotz [8] and, more recently, by Wells.[9] The latter stated that the changes in the physicochemical properties of arteries support the view that arteriosclerosis depends primarily on the reduction in elasticity of the media, and that the subsequent changes seem to be due to the yielding of the media. To support this theory numerous lesions have been described as occurring in the media; among these can be listed swelling, fragmentation, diminution in volume, fatty infiltration, and calcification of elastic fibers. The problem is apparently complicated by the belief that there is considerable diversity in the vascular manifestations, depending upon the site and type of vessels involved. Thus, Winternitz, Thomas and LeCompte [10] have recently restated a generally accepted concept that "the muscular arteries, for example, are very prone to undergo medial change ending in calcification, without necessarily involving either the intimal or adventitial coats, whereas this type of change in the larger elastic arteries is rare."

However, calcification of the media of the aorta has been observed by Klotz,[8] Ribbert,[11] Ravault,[12] Farkas and Fasal,[13] as well as by Faber.[7] Of these investigators, only Faber believed that there was a relationship between calcification of the media of the aorta and the production of intimal arteriosclerosis; the others could find no direct parallelism between the amount of calcium deposited in the media and arteriosclerosis of the intima. In all these investigations it was recognized that calcification of the media rarely occurred in young persons, but no organized attempt was made to correlate this change with age. It would thus appear that while calcification of the media of the human aorta has been recognized, little is known of its significance. Faber, as well as Farkas and Fasal, attempted to study calcification of the media of the aorta and its relation to ageing and disease, but they were unable to reach any definite conclusion from the limited number of cases studied.

In the course of study of human aortas from routine autopsies, we noted the frequent occurrence of a blue-staining, finely granular material in the media of the aorta which was apparently similar in character to that described by Björling [14] as mucoid degeneration. However, subsequent analysis by micro-incineration showed this material to be composed largely of calcium, and on the basis of this observation it was decided to investigate the relationship of calcification of the media of the aorta to intimal arteriosclerosis, ageing and disease.

Materials and Methods

A preliminary experiment was carried out in an attempt to determine whether the dark blue granular material in the media of aortas stained with alkaline Delafield's hematoxylin was calcium. Sections were made from 10 aortas imbedded in paraffin in the usual manner. These were mounted on slides for study by micro-incineration, and control slides were stained with alkaline Delafield's hematoxylin and eosin. The technic of micro-incineration was the same as that developed by Scott,[15] and also employed by one of us (Lansing).[16] Examination of these incinerated preparations by darkfield microscopy revealed areas containing large amounts of white ash which corresponded in general to the areas of dark blue granular material in the hematoxylin and eosin preparations. This white ash has been identified clearly as calcium by Lansing and Scott.[17] That the blue granular material represents calcium is also indicated by the observation that this staining reaction can be prevented by decalcification in dilute nitric acid (Figs. 7 and 8).

In a second experiment 50 specimens of aortas of various ages taken from the proximal part of the arch, and showing varying degrees of blue-granular material in the media, were examined also by the technic of micro-incineration. It was noted that the areas showing the greatest concentration of calcium in the micro-incinerated preparations corresponded to the sites of deposition of the blue-granular substances in the hematoxylin and eosin preparation. There were, however, other areas in the micro-incinerated preparation in which there was more finely dispersed calcium, and these could not be identified in the hematoxylin and eosin preparations. This indicates the greater sensitivity of the method of micro-incineration for the demonstration of calcium.

With the previous two experiments as a basis, a statistical analysis was carried out for the purpose of determining the relationship of age and disease to calcification of the media. For this purpose we used specimens from 582 aortas, removed from the proximal portion of the arch and stained with alkaline Delafield's hematoxylin and eosin. This

number included the 60 specimens used in the two preliminary experiments. It should be stated that in those cases in which the hematoxylin and eosin stain failed to reveal evidence of calcification of the media, either micro-incinerated preparations were made or the slides were restained. This was done because it was noted that when the hematoxylin became old, or had lost some of its alkalinity, it failed to reveal the presence of the blue-granular material in the media. Also, in some cases in which autopsy had been performed 18 or more hours after death, even fresh hematoxylin preparations failed to show evidence of calcium. In all of these cases, however, the presence of calcium in the media could be demonstrated in micro-incinerated preparations.

The sections were arbitrarily graded as negative, 1 plus, 2 plus, or 3 plus (0, +, ++, or +++) in accordance with the degree of calcification of the media; photomicrographs illustrating these grades, both by micro-incineration and by hematoxylin and eosin staining, are shown in Figures 1 to 9.

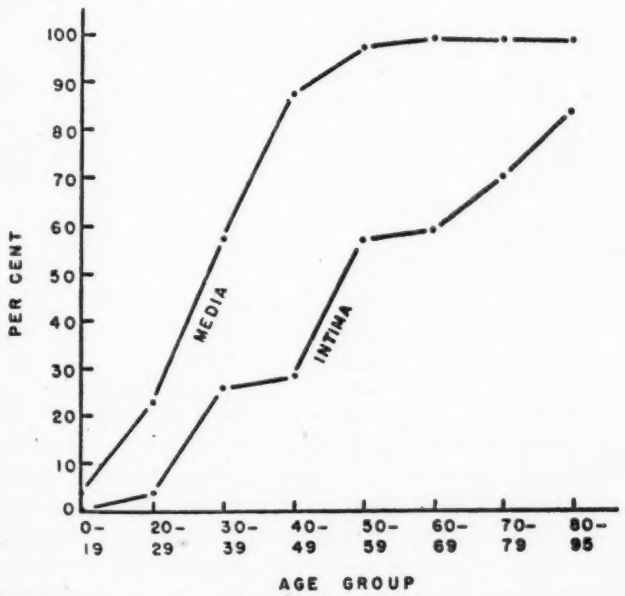
In a final experiment various levels through the whole length of 6 aortas were studied in order to determine whether or not the intensity of calcification of the media is uniform throughout the length of the aorta, as well as to determine if there is any correlation between the intensity of calcification of the media and the site of formation of intimal plaques.

Results

In 60 aortas we carefully compared the site of deposition of the blue-granular material in hematoxylin and eosin sections with the areas of calcification in corresponding micro-incinerated preparations. In those showing a slight degree of calcification of the media, it could be seen that the calcium was deposited between the muscle fibers; this distribution would correspond to the areas usually occupied by elastic fibers. With progressive increase in the amount of calcium, the areas involved became more extensive, and in pronounced cases the cellular structure of the media became entirely obliterated. In the latter instances it was possible to conclude that both muscle and elastic fibers were heavily infiltrated with calcium. This can be seen in Figures 7 and 9. Occasionally in senile specimens discrete concretions of calcium were observed in addition to the general 3 plus calcification of the media. However, such condensations occurred relatively infrequently.

Specimens taken through various areas of 6 whole aortas, from subjects ranging in age between 35 and 83 years, were studied by micro-

incineration. The areas studied included the proximal part of the arch free of intimal plaques, the abdominal aorta free of intimal plaques, and the abdominal aorta including intimal plaques. In general, the intensity of calcification of the media was greater in the abdominal than in the thoracic portion of aortas taken from subjects 35, 55 and 63 years old. In the remaining 3 subjects, 70, 76 and 83 years old, the concentration of calcium in the media was so great throughout the aorta that it was not possible to estimate quantitative differences between the thoracic and abdominal portions. In the three younger cases it could be determined also that the concentration of calcium in the abdominal portion of the aorta was greater in the area immediately under an intimal plaque than where there was no evidence of intimal



Text-Fig. 1. The age-incidence of medial calcification and of intimal sclerosis, based on 540 aortas.

proliferation, while in the three older specimens the concentration of calcium throughout the media was so great that such a difference was no longer discernible.

The age-incidence of calcification of the media obtained from the examination of 540 aortas of various ages is shown graphically in Text-Figure 1. Prior to 20 years of age medial calcification occurred in only 4 per cent of the cases; between 20 and 30 years this increased

to 58 per cent; between 40 and 50 years 98 per cent showed this change, and after 50 years of age calcification of the media was present in all cases exclusive of certain instances in which there were other diseases of the media. It should be noted that these figures do not take into account the intensity of calcification of the media; they present only the incidence. The data in Table I show that the intensity of calcification of the media as well as the frequency of its occurrence increases with age. Thus, calculations from the data in this table show that prior to 50 years of age 56 per cent of the cases showed no medial calcification, 32 per cent showed slight (+) calcification, and only 12 per cent showed marked calcification (++ and +++). After 50

TABLE I

Age Distribution and Correlation of Calcification of the Media and Intimal Proliferation in the Human Aorta*

Age groups	No intimal lesions				With intimal plaques				Calcified plaques			
	0*	1	2	3	0*	1	2	3	0*	1	2	3
0–19	47	2				1						
20–29	20	4	1			2	4			3		1
30–39	16	12				2	3	1		5	2	
40–49	5	20	7		1†	14	11	3	1	10	6	5
50–59		23	11	3		14	13	6		21	20	7
60–69		32	15	7		8	10	4		22	22	14
70–79		20	9	3		1	3	1		13	11	11
80–95		4	2	1								

* Medial changes are graded 0, 1, 2, and 3 in accordance with the degree of calcium deposition.

† Connective tissue penetration of the media.

years of age there were no cases free of this change, while 48 per cent had slight calcification (+) and 52 per cent showed a marked deposition of calcium in the media (++ and +++). The influence of age on the intensity of medial calcification is shown also in the first two columns of Table II, where it can be seen that there is in both sexes a progressive increase in the mean intensity of calcification with increasing age, at least up to 59 years. Apparently the increase with age is less definite after 60 years.

In Table II we have analyzed statistically the intensity of medial calcification of the aorta on the basis of sex and of various diseases of which there were sufficient cases to allow for the determination of significant differences. There is no significant difference between the intensity of calcification of the media in corresponding decades in the two sexes. The cases in which death was due to a rapidly fatal accident or to an acute infectious disease were used as controls since these would not be expected to have an influence on calcification of the

TABLE II

The Influence of Sex and Disease on the Graded Incidence of Deposition of Calcium in the Media of the Aorta as Correlated with Age

Classification	Age groups							
	0–19	20–29	30–39	40–49	50–59	60–69	70–79	Over 80
Females (all cases)	0.0 ± 0.0	0.4 ± 0.2	0.8 ± 0.2	1.2 ± 0.1	1.7 ± 0.3	1.6 ± 0.1	1.7 ± 0.1	1.8 ± 0.2
Males (all cases)	0.1 ± 0.1	0.1 ± 0.1	0.6 ± 0.2	1.2 ± 0.1	1.5 ± 0.1	1.7 ± 0.1	1.8 ± 0.1	2.1 ± 0.2
Accident or acute disease	0.0 ± 0.0	.....	.....	1.0 ± 0.2	1.2 ± 0.1	1.6 ± 0.1	2.0 ± 0.2	1.9 ± 0.3
Tuberculosis	0.1 ± 0.1	0.3 ± 0.2	0.6 ± 0.1	1.2 ± 0.3	1.4 ± 0.2	1.6 ± 0.2	1.9 ± 0.2	.....
Cancer	.....	.....	1.0 ± 0.6	1.1 ± 0.2	1.8 ± 0.1	1.8 ± 0.1	1.3 ± 0.1	2.3 ± 0.3
Nephrosclerosis and/or hypertension	.....	.....	2.8 (4)	1.6 ± 0.2	1.8 ± 0.3	1.5 ± 0.1	1.5 ± 0.2	1.8 ± 0.3
Coronary thrombosis, cerebral thrombosis and hemorrhage	.....	.....	.....	1.3 (4)	1.6 ± 0.2	1.7 ± 0.2	1.9 ± 0.2	2.1 ± 0.4
Syphilitic aortitis	.....	.....	0.0 (3)	0.0 ± 0.0	0.2 ± 0.1	0.4 ± 0.2	0.1 ± 0.1	.....

Figures in parentheses represent the number of cases when they were relatively few.

media. When this group is compared with the two groups according to sex, it may be noted that the mean values for the three groups are essentially the same for corresponding age groups except for the decade between 50 and 60 years. Here the mean value for the group with accidental death is somewhat lower than those generally obtained in males and females of corresponding age; this difference, however, is of doubtful statistical significance and may be due to the effects of other diseases included in the two groups according to sex, as will be noted later. A comparison of the data from patients dying of tuberculosis with those obtained from groups with deaths from accident and acute diseases does not reveal any significant differences. There were no patients below 30 years of age dying of cancer. The mean values of the various age groups of cancer patients, when compared with the group with accidental death, were essentially similar except during the decades of 50 to 60 and 70 to 80 years; in the former decade the mean intensity of calcification in cancer patients was greater, while in the latter it was lower, than in the group with accidental death. Another group was composed of patients dying of coronary arterial thrombosis and cerebral arterial hemorrhage or thrombosis. As would be expected, there were none younger than 40 years of age in this category, and only 4 in the decade from 40 to 50 years. There were no significant differences betwen this group, the group with accidental death, or the groups according to sex.

The most striking results were obtained from a comparison of the intensity of medial calcification in subjects with syphilitic aortitis and all other groups. Of 42 specimens of syphilitic aortitis studied, 33 showed no calcification of the media and only 9 showed it to a very slight degree (+). Characteristic of these cases was partial replacement of the media by fibrous tissue; an example of this is shown in Figure 10. It may be noted here that there were two other cases with connective tissue penetration into the media which did not show the inflammatory change characteristic of syphilitic aortitis and in which no definite etiology could be established; here also no evidence of calcification of the media could be obtained. In Figure 11 an incinerated preparation of typical syphilitic aortitis is shown; despite the advanced age of this subject (72 years), the calcium content of the media is essentially comparable to that of a child (*cf.* Fig. 2).

Discussion

The term "arteriosclerosis" has generally been used to describe various changes in the arteries which lead to their loss of elasticity. In the aorta it denotes a thickening of the intima produced by a new forma-

tion of connective tissue in which there may be found small and large wandering cells laden with fat. In later stages the thickened intima becomes hyaline or necrotic about the more abundant accumulations of fat, and secondary areas of calcification may occur. Medial changes are usually held to be the result of encroachment of the intimal plaques upon the media. Although several investigators have described the frequent occurrence of calcium deposits in the media of the human aorta, this phenomenon has not received general recognition, probably due to a tendency to look for gross or relatively homogeneous concretions such as the calcium plaques which occur in the intima of the aorta and in the media of muscular arteries (Mönckeberg's sclerosis). Calcification of the media of elastic arteries has been described (see Introduction) as being essentially microscopical and has been observed only by the use of special stains or micro-incineration. However, we have observed in a few cases extreme calcification of the media leading to the formation of concretions. From a physiological point of view, the degree of calcification observed in these experiments can be expected to produce functional changes in the contractility of the aortic wall.

Our studies tend to confirm the observations of Ravault [12] that calcification of the media of the aorta is primarily associated with the elastic elements, since in the early stages calcium is deposited between muscle fibers rather than inside the muscle cell. It is of interest in this respect that in syphilitic aortitis, in which the elastic elements of the media are destroyed, the amount of calcium is markedly less, or it may be absent. In more severe changes, the calcium becomes generally deposited and involves all of the elements composing the media. However, Hass [18] has observed the formation of collagenous splints in the media, and the deposition of calcium in these structures as well as in the elastic lamellae must be considered as a possibility.

It is interesting to note that Shelling, Asher and Jackson,[19] as well as Johnson,[20] have observed calcified and degenerative arterial lesions in human beings with adenomas of the parathyroid glands, and similar changes have been described in dogs following the administration of large amounts of parathyroid hormone (Hueper,[21] Learner,[22] McJunkin, Tweedy and Breuhaus [23]). While the experimental lesions mainly involve the media, in man the intima is affected as well as the media. A more extensive lesion involving both the intima and media of elastic as well as muscular arteries has been produced in rabbits, cats, dogs and rats by the administration of large amounts of vitamin D (Kreitmair and Hintzelmann,[24] Schmidtmann,[25] Hueper,[26] Ham and Lewis,[27] Ham and Portuondo,[28] Shohl, Goldblatt and Brown,[29] and

others). Hueper [26] has observed similar results following a lengthy exposure of rats to ultraviolet irradiation. It should be noted that in these lesions there are areas of intimal proliferation in close proximity to the areas of calcification of the media.

We have seen in the present observations a step by step increase in the amount of calcium deposited in the media of the aorta with increasing age. Wilens [5] has demonstrated that in the human aorta the elasticity progressively decreases with age and is primarily dependent upon changes in the elastic elements in the media. Similarly, Saxton [30] has studied the extensibility of the rabbit aorta with respect to age and has been able to show that this vessel becomes more extensible with increasing age; it also becomes less able to return to its original circumference after stretching. However, the results of this investigator suggested that the rabbit aorta does not age as rapidly as the human aorta and is still a relatively young structure at the end of the life span of this species. Hass [18] has used purified elastic tissue preparations, and has observed that the extensibility of purified networks of elastic tissue is greatest in young specimens and usually decreases with age, although occasional aged elastic systems have the characteristic high extensibility of youthful tissue. Unfortunately, it cannot be ascertained from Hass' experiments what rôle calcium plays in this change in extensibility because the method used in the purification of elastic tissue removes the calcium from this tissue. However, it is very likely that with the infiltration into the media of large amounts of calcium with age there would be a profound diminution in both elasticity and contractility of the aorta. Significantly, the curve of reduction of elasticity with age, as demonstrated by Wilens,[5] closely parallels the curve of intensity of calcification of the media with age in the present experiments.

As stated previously, the theory that loss of elasticity and weakening of the vessel wall are the primary factors which precipitate the formation of subsequent intimal lesions has been considered for a long time. The chief supporters of this concept have been Thoma and Kaefer,[6] Faber,[7] Klotz,[8] Wells,[9] and, more recently, Wilens [5] and Hass.[18] The latter investigator believes that "the intimal changes represent a fortuitous accumulation of lipids in a collagenous splint that is deposited in response to a primary failure of medial systems to maintain integrity of function in the presence of imposed tensions." Most of the theories concerning the focal nature of early atheromatous plaques are based on the assumption that there are certain sites subject to particular stresses and strains. Some investigators such as Duguid [31] and Moschcowitz [32] believe that this leads to a localized impairment of elasticity

followed by fibrous overgrowth of the intima at that site, or to a wrinkling with formation of connective tissue interstices. Other investigators stress primarily the intimal changes. Thus Aschoff [33] described swelling of the ground substance, while Krafka [34] observed separation or actual intimal herniation. Moon [35] placed emphasis on a local anemia, while Winternitz, Thomas and LeCompte [10] described in great detail the increased intimal vascularity and hemorrhage. It is particularly the latter group of investigators who have demonstrated the rich network of small vessels which supply the arterial wall; some of these are derived from the vasa vasorum in the adventitia, while others have their origin as tiny openings in the endothelial lining of the aorta. These vessels are generally composed of a single layer of endothelial cells and depend upon the contraction of the aorta to propel their contained blood. It then becomes apparent how a change in the elasticity and contractility of the aortic wall would influence the rate of flow of blood through these small vessels. A diminution in contractility could account for a hemostasis which would result in small thrombi and hemorrhages which Winternitz, Thomas and LeCompte described as the earliest changes in the formation of atheromatous plaques; it would also account for a diminution in the amount of oxygen supplied to the intima to which Hueper [26] attributes importance in the formation of intimal plaques. Recently Wilens [36] has immobilized segments of vessels by placing silver cuffs about the femoral and carotid arteries of rabbits. This produces an adventitial thickening and fibrosis, and a thinning of the media due to atrophy of smooth muscle and condensation and fragmentation of elastic fibers. He then observed that cholesterol feeding usually leads to a selective localization of lipids in the intima of arteries at the region of the cuffs. Two observations in the present series may be in essential agreement with the interpretation that local immobility may influence the site of formation of intimal plaques. Although we have studied only 6 complete aortas, it appears that the abdominal portion undergoes a more pronounced degree of calcification of the media than does the thoracic segment; it is generally agreed that the intimal involvement in arteriosclerosis is more extensive in the abdominal than in the thoracic part of the aorta. In addition, in 3 cases we have observed that within a single aorta calcification of the media is more intense in the immediate vicinity of intimal plaques than elsewhere. The first of these two observations is contrary to a previous observation by Ravault [12] who noted a higher degree of calcification in the thoracic than in the abdominal portion of the aorta.

The assumption that in the aorta medial involvement is secondary to intimal changes is without support if the present results are valid. It

would appear more likely that intimal plaques are a result of medial injury plus, probably, other localized conditions. This contention is supported by the observations that calcification of the media precedes the formation of intimal plaques and that intimal plaques do not occur without medial calcification or some other injury to the media.

The present experiments also show that calcification of the media of the aorta is apparently not influenced by sex and by most of the diseases analyzed in our statistical data. Chronic disease *per se* apparently has no influence on this change. However, the mechanism of calcification of the media may be influenced by certain diseases. Thus, in the analysis of the 42 cases of syphilitic aortitis, 33 cases show no evidence of calcification, while in 9 cases there was only a mild degree of this change. Characteristic of syphilitic aortitis is the breakdown of the muscular and elastic elements of the media and the infiltration of scar tissue. The end-product of syphilitic aortitis, namely, the scarring of the media, results in a disruption of the normal vasa vasorum as well as a loss of contractility and elasticity. This can be expected to produce an effect upon the intima comparable to that of calcification of the media; and, indeed, thickening of the intima is a common finding in syphilitic aortitis, although the plaques may not become calcified as readily as those seen in arteriosclerosis. Also characteristic of syphilitic aortitis is the fact that plaques are more concentrated in the thoracic portion of the aorta, and therefore it would be of interest to determine if there is any difference in the degree of scarring and decalcification between the thoracic and abdominal portions of the aorta. This we hope to do in future experiments.

The data on hypertension lead to the interesting observation that calcification of the media of the aorta in the third, fourth and possibly the fifth decades is more pronounced than in the other diseases studied. It cannot be stated at present whether this is the effect of an existing hypertension or whether it is a factor in the etiology of the latter disease. However, it is conceivable that calcification of the media of the renal artery resulting in relative hemostasis could have an effect similar to that of the Goldblatt kidney. This also must await further investigation.

We have seen, therefore, how calcification of the media of the aorta may play a specific rôle in the genesis of arteriosclerosis. But the present observations also have a broader biological significance, namely, their relationship to processes of ageing in general. The present work demonstrates that the variations in frequency of calcification of the media as well as in the intensity of this phenomenon are correlated with ageing. The increase in calcium deposition in the soft tissues in human

beings is entirely in line with the fact that the calcium concentration increases with age in a variety of tissues and organs of various species. (See Lansing [16] for a review of the literature on this subject.) Calcification of the media of the aorta, in particular, has been seen in a wide variety of lower mammals and birds by Fox.[37] Barnes [38] has observed that in rats the incidence of calcification of the heart is an increasing function of the age at death. It has been suggested by Lansing [16] that this increase in calcium is a general characteristic of the ageing process. He has demonstrated that calcium increases with age not only in the vertebrates but also in invertebrates and even in plants. It is interesting to note that the age-intensity curve of medial calcification of the aorta (Table II) closely parallels the age-calcium concentration curve which he obtained on the water plant, Elodea. It appears, therefore, that we are dealing with a fundamental process in the biology of ageing, which in the specific instance of the aorta may result in secondary intimal changes producing the phenomenon of arteriosclerosis.

Summary

The frequency of occurrence and the influence of age, sex and disease on calcification of the media of the human aorta were studied by means of sections prepared by hematoxylin and eosin staining and by micro-incineration. The results showed that calcification of the media precedes the formation of intimal plaques; that medial calcification occurs more frequently than do intimal plaques; that intimal plaques do not occur without calcification of the media or other medial change such as syphilitic aortitis, or marked connective tissue infiltration of the media; and that within a single aorta medial calcification is probably more intense in the immediate vicinity of an intimal plaque than elsewhere. In a few observations it was noted also that calcification of the human aorta was more pronounced in the abdominal than in the thoracic portion of the aorta.

Calcification of the media of the aorta was shown to be primarily a function of age and was not influenced by sex and various chronic infectious diseases. However, specimens from hypertensive persons between the ages of 30 and 60 years showed considerably more medial calcification than did the "controls." Of 42 cases of syphilitic aortitis, 33 showed no medial calcification and 9 showed only slight calcification of the media.

The relationship between calcification of the media of the human aorta and the loss of elasticity and contractility with age, as well as the possible relationship of these changes to the formation of intimal plaques, is discussed.

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[*Illustrations follow*]

DESCRIPTION OF PLATES

PLATE 131

FIG. 1. Section of the aorta of a 14-year-old male stained with hematoxylin and eosin. There are no abnormal deposits in the media. × 90, thickness 4 μ.

FIG. 2. Section of the aorta of a 14-year-old male prepared by the microincineration technic. The white ash represents deposits of calcium which correspond to the location of the nuclei in hematoxylin and eosin specimen. × 90, thickness 4 μ.

FIG. 3. Section of aorta of a 40-year-old male stained with hematoxylin and eosin. A few plaque-like deposits in the media can be seen. This illustrates a 1 plus (+) deposition of calcium. × 90, thickness 4 μ.

FIG. 4. Section of aorta of a 38-year-old male prepared by the microincineration technic. The deposits of calcium in the media are larger than those seen in Figure 2. This illustrates a 1 plus (+) deposition of calcium. × 90, thickness 4 μ.

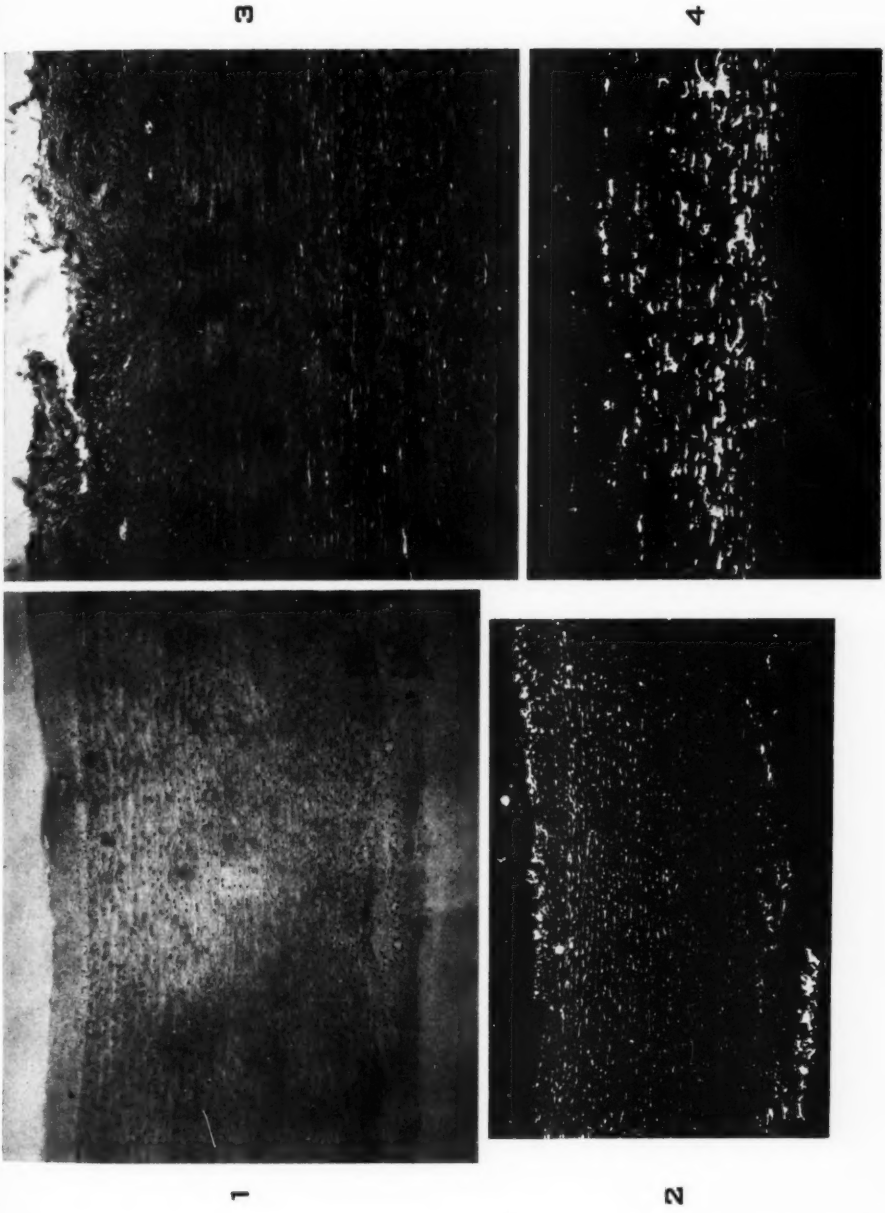

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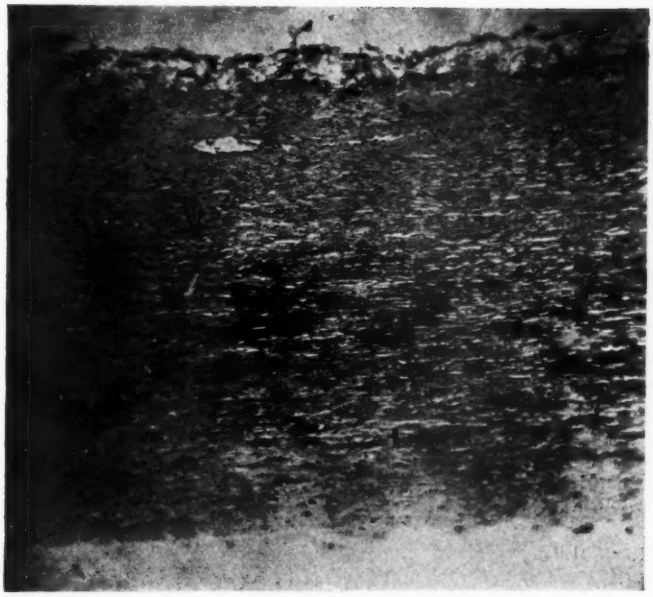
PLATE 132

FIG. 5. Section of aorta of a 53-year-old male stained with hematoxylin and eosin. There is an increase in the number and size of calcium deposits over those seen in Figure 3. This illustrates a 2 plus (++) deposition of calcium. × 100, thickness 4 μ.

FIG. 6. Section of aorta of a 49-year-old male prepared by the microincineration technic. The deposits of calcium are large and confluent. This illustrates a 2 plus (++) deposition of calcium. × 100, thickness 4 μ.

5



6

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PLATE 133

FIG. 7. Section of aorta of an 80-year-old male stained with hematoxylin and eosin. A large confluent area of calcium in the media can be seen. This illustrates a 3 plus (+++) deposition of calcium. × 100, thickness 4 μ.

FIG. 8. Same specimen as that shown in Figure 7, after decalcification with dilute nitric acid. This illustrates the removal of the blue-granular material from the media by decalcification.

FIG. 9. Section of aorta of a 75-year-old male prepared by the microincineration technic. This demonstrates a marked deposition of calcium in the media, and represents a 3 plus (+++) deposition of this substance. × 100, thickness 4 μ.

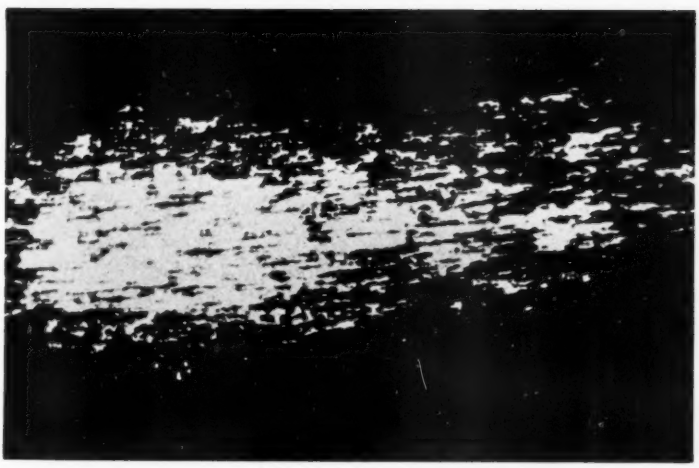

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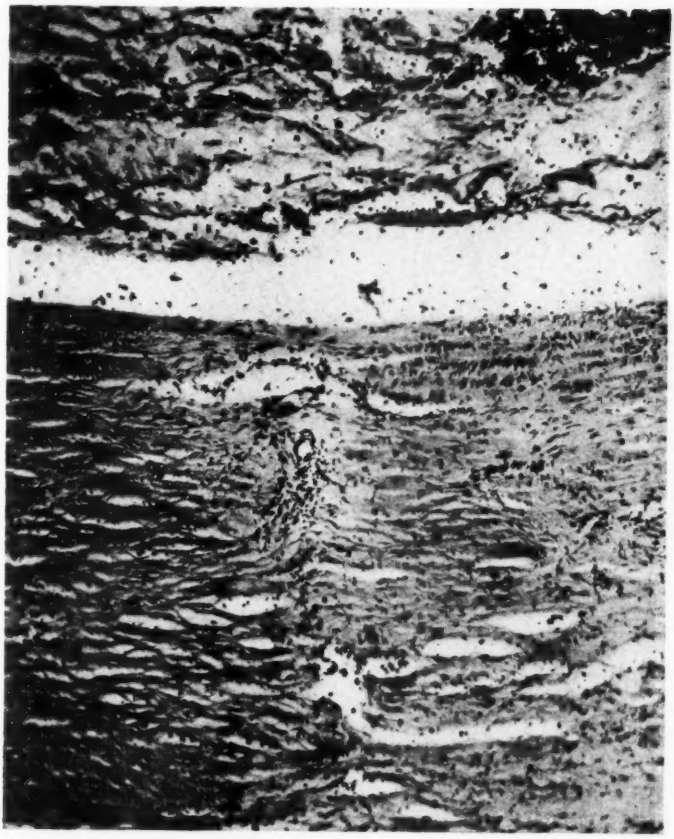
PLATE 134

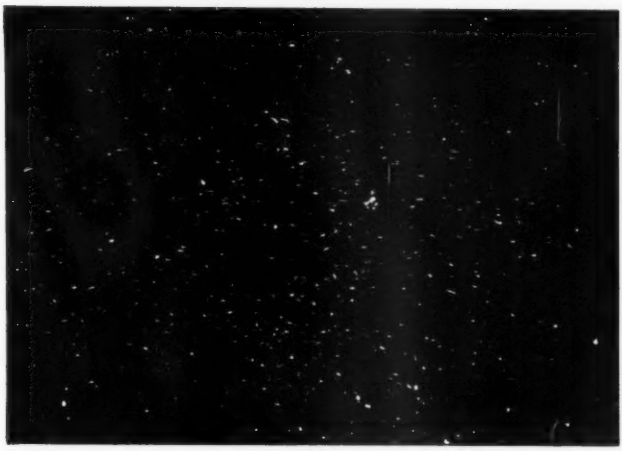
FIG. 10. Section of aorta of a 72-year-old male stained with hematoxylin and eosin. There is a complete absence of calcareous patches in the media. A transverse medial scar is present. The adventitia, infiltrated with lymphocytes, is at the top of the figure. × 100, thickness 4 μ.

FIG. 11. Same specimen as that shown in Figure 10 prepared by the microincineration technic. A marked diminution in calcium content of the media is apparent. × 100, thickness 4 μ.

10



11



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CUSHING'S SYNDROME WITH POSSIBLE PHEOCHROMOCYTOMA

Report of a Case *

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The following case is reported because it represents a clear-cut example of "pituitary basophilism" without adenoma of the pituitary gland but with a tumor of the adrenal of uncertain histogenesis, perhaps of medullary origin.

Report of Case

Clinical History. The patient (F. S. H. no. 3289), was a housewife, 31 years old, of German ancestry who was admitted on June 20, 1942. There was a family background of mental disease; the maternal grandfather and a maternal aunt had died in psychiatric hospitals, another maternal aunt had had a "nervous breakdown," and a sister had been hospitalized since 1938 with a diagnosis of dementia praecox, catatonic type.

Nothing unusual was elicited concerning the patient's early life. She was married in 1937. For 3 years, beginning in 1936, she had had several attacks of renal colic and passed some stones. Her menstrual periods had begun at age 14 but since the age of 20 had been occurring only every 2 or 3 months and the flow had been scanty. Endocrine therapy had no apparent effect. Some months after her marriage she began to put on weight and to grow a beard. In January, 1942, her local physician found her "literally swollen like a balloon," apparently from edema. She was hospitalized and the swelling subsided somewhat, leaving striae on the abdomen. At this time urinary symptoms were indefinite. She was found to have a blood sugar ranging around 150 mg. per cent and was put on a diabetic diet with insulin. She was started on 35 units of insulin, but this seemed to be too much, and the dose was progressively cut to 15 units daily. She was given stilbestrol for her amenorrhea, which was now complete, without effect. Following discharge from the hospital she apparently had several insulin shocks. She had a persistent hypertension. For some months she was under treatment for vaginitis (*Trichomonas vaginalis*) by her family physician. He felt sure that, during the few years the patient was under his care, the clitoris had about doubled in size, reaching finally a diameter of about 1 cm. On June 14, 1942, he was called to see the patient, who was apparently in a state of insulin shock. Her systolic blood pressure was 130, and diastolic, 80, unusually low for her, and she talked in a confused manner. After admission to the Danbury Hospital she became noisy, talked about machines working on her, and showed alternately a ravenous appetite and a disinclination to eat. Frequently she would seize food from a tray and bolt it without chewing. At times she ran screaming back and forth through the halls and on one occasion tried to jump from a window. Her blood pressure was 150/90 on several occasions, and at one time rose to 174/120. The blood sugar ranged from 120 to 182 mg. per cent. She was put on a regime of 25 units of protamine zinc insulin before breakfast, and this was later increased to 35 units.

On admission to the Fairfield State Hospital on June 2, 1942, she showed a masculine distribution of hair, with hirsutism of face and body, obesity confined

* Received for publication, July 29, 1943.

to the head, neck and trunk, a peculiar moon-like, purplish, cyanotic appearance of the face, acne of face and body, and moderate exophthalmos. There were many purplish striae on the abdomen. Blood pressure was 150/100. Abdominal reflexes were absent, the gait was slow and on a wide base, and there was a suggestive Romberg sign. The ocular fundi appeared negative.

The patient seemed uninterested in her appearance and was apathetic. She wandered about aimlessly, with shuffling gait and arms hanging at her sides. Occasionally she assumed an almost catatonic posture, with her hands in her lap and eyes immobile. She answered questions fairly coherently, but occasionally spoke of a machine working on her.

Both remote and recent memory were fairly well preserved. She repeated seven digits forward but was unable to repeat any backward. Answers to simple arithmetical problems were fairly accurate but delayed. She said that ever since her menstrual periods stopped in January, 1942, she had been unable to concentrate.

The patient continued to be somewhat confused. Shortly after admission she complained of sudden pain in the right chest, and her temperature rose to 101.2° F. A roentgenogram revealed increased density, obscuring the lower portion of the right lung, and, in the upper thorax, multiple healed fractured ribs on both right and left. On June 30, 1942, an indurated area in the right axilla was drained, yielding pus which showed on smear gram-positive cocci and gram-negative rods. On July 2, 1942, the temperature rose to 105° F. She complained of dyspnea. On July 4, 1942, she died rather suddenly, a final temperature of 107.2° F. being recorded.

Laboratory Findings

Urine

	Specific gravity	*Albumin*	*Sugar*	*Acetone*	*Sediment*
6/25/42	....	....	Negative	Negative	....
6/30/42	1.012	++	+	....	15–20 white blood cells per field; 3–10 red blood cells per field; *Trichomonas vaginalis*
7/2/42	....	....	++++	Negative	
7/4/42	....	....	++	Negative	

Blood Cells

	*Red blood cells**	*White blood cells*	*Polymorphonuclear leukocytes*	*Lymphocytes*	*Mononuclear leukocytes*	*Smear*
6/22/42	4,400,000	7,900	83% (segmented, 77%; nonsegmented, 6%)	11	6	
7/2/42	....	9,300	91% (segmented, 69%; nonsegmented, 22%)	4	1	3 metamyelocytes, 1 myelocyte

Blood Chemistry

	Sugar	*Nonprotein nitrogen*
6/22/42	148 mg. %	32 mg. %
6/25/42	99 mg. %	
7/1/42	131 mg. %	28 mg. %
7/3/42	159 mg. %	32 mg. %

* Hemoglobin, 14.5 gm.

Additional Tests

6/24/42 Kline exclusion test, negative.

7/1/42 Basal metabolic rate, +6 and +7 (two readings). (Patient had a slight fever on this day.)

Necropsy Findings

The post-mortem examination was started about 10 hours after death. The body measured 159 cm. and weighed about 120 lbs. The general configuration was striking. The head, neck and trunk had a puffy appearance due to the massive deposition of subcutaneous fat, but the extremities were thin, the fingers and ankles being slender and delicate. A dusky cyanosis of head and neck, extending down to the clavicles, had persisted. Indurated, papular, acneform lesions were present on the face and anterior part of the body. The eyes were prominent and there was reddening of the conjunctivae. The hirsutism consisted not only of a masculine distribution of the pubic hair, which extended up to and beyond the umbilicus in the midline, but also a growth of coarse, long, yellow-brown hair over the well developed breasts, over the lateral aspects of the abdomen, over the chin and upper part of the neck, and over the upper lip. The hair of the scalp and axillae was abundant. The abdominal striae were irregular purplish bands, 6 or 8 on each side, 4 or 5 mm. wide, extending from the groin to a level somewhat above the umbilicus.

The right pleural cavity contained about 700 cc. of slightly cloudy, yellow fluid containing flecks of fibrin. The *heart* weighed 380 gm. and showed moderate thickening of the wall of the left ventricle. The *lungs* were congested and boggy; each pulmonary artery contained a friable red-brown embolus, and in the right lower lobe a softened, opaque, brownish infarct was encountered. No source was found for the emboli. The *spleen* weighed 150 gm., had a tense capsule and a soft, friable, bulging pulp. Microscopically, there was marked congestion and a rather striking paucity of lymphocytes in both the malpighian corpuscles and the pulp. The *pancreas* weighed 80 gm.; microscopically both the acini and islets were well preserved. The *kidneys* weighed 137 and 185 gm., respectively; they were rather pale and slightly swollen, with no gross evidence of scarring. In the left ureter, at its origin from the pelvis, there was a soft, yellow, crumbling calculus about 2 cm. in diameter. This material did not burn in the flame and dissolved in hydrochloric acid with effervescence, hence was probably chiefly calcium carbonate. Microscopically, the renal tubules were poorly stained and granular, doubtless because of post-mortem change. The large and medium-sized arteries showed no definite

change, but many of the afferent arterioles at their entrance into the glomerular tuft showed a distinct hyaline thickening (Fig. 6). This change was quite diffuse, being found throughout both kidneys. The *uterus* measured 7 by 4 by 3 cm.; it showed a smooth, pink endometrium and firm muscular wall.

Each *ovary* was small (about 2.5 by 1 cm.), hard and yellow-white, like that of a senile woman. In the left was a cyst 1 cm. in diameter containing a clear fluid. Microscopically there were several corpora albicantia but no corpora lutea; a number of primordial follicles were seen, and one questionable graafian follicle. The *thyroid* weighed only 10 gm.; under the microscope the acini varied considerably in size and were lined by a cuboidal epithelium of moderate height, without papillary infolding; colloid was fairly abundant and showed vacuolation. The *parathyroids* did not appear remarkable grossly. The *bones* were cut with unusual ease and showed a sparse trabecular pattern. The *bone marrow* showed a rather marked shift to the left in the granulocyte series, as might be expected from the last blood count. The major *arteries* were almost totally free from atheromatous change. A section of *skin* taken through one of the abdominal striae showed a slight thickening of the epidermis and partial replacement of the dense collagenous tissue of the corium by an accumulation of fairly young fibroblasts arranged parallel to the surface. An elastic tissue stain showed no definite diminution of the elastic fibers. In spite of the purple color seen during life, no abundant vascularity was seen in the section (a similar experience is recorded by MacCallum, Futcher, Duff and Ellsworth[1]). The brain weighed 1300 gm., was symmetrical, and showed no narrowing or flattening of the convolutions. Blocks from the anterior and posterior hypothalamus, frontal cortex, hippocampus, cerebellum and medulla, embedded in celloidin and stained by a modified Nissl's method,* showed no alterations in the ganglion cells or other changes. Sections taken in the vicinity of the infundibulum did not show the proliferation of large vessels described by Globus[2] in a case of Cushing's syndrome.

The region of the *right adrenal gland* was occupied by a mass of neoplastic tissue weighing 183 gm. The lower portion of this mass was the better preserved part and had a fairly well defined capsule of fibrous tissue. The cut surface was rather soft and of a deep yellowish brown color, with fine streaks of gray. Some of the tissue, near the capsule, was red-blue and boggy. The upper and larger part of the tumor mass was firmly attached to the under surface of the liver and

*The brain tissue was fixed in a 4 per cent solution of formaldehyde, embedded in celloidin and stained with toluidine blue.

had a delicate capsule which was easily ruptured, allowing the escape of markedly autolyzed, soupy, yellow-brown tissue. When the right suprarenal vein was opened, a portion of the tumor was seen to be bulging up beneath the endothelium of the vein. It had not broken through, however, and no metastases were found. A small amount of the right adrenal, showing cortex and medulla, was found attached to the medial border of the tumor mass.

Microscopically, much of the neoplastic tissue was necrotic, and only poorly stained outlines of cells were seen. In the better preserved parts, the cells assumed in places a rather compact arrangement of relatively small cells in sheets or masses separated by large sinusoidal blood vessels (Fig. 1). In such regions there was often a suggestion of a cord-like arrangement but no definite resemblance to any part of the normal adrenal cortex. (Fig. 7, from a large, apparently inactive cortical adenoma discovered at autopsy in another patient, is included for comparison.) In other places the cells had a still more haphazard arrangement and were larger, varying in shape and size, with abundant acidophilic cytoplasm and occasional bizarre giant nuclei (Figs. 2, 3 and 4). Vacuolation of the cytoplasm was not prominent, most of the cells having a granular appearance. Mitotic figures were extremely rare. In a few cells there was a fine yellow-brown pigment. This occurred chiefly in cells surrounding some of the large sinusoids (Fig. 5). It appeared only in the tissue which had been fixed in Zenker's fluid, not in that which was fixed in formaldehyde solution.

In an attempt to decide definitely whether the tumor was of cortical or medullary origin, use was made of the histochemical methods described by Bennett.[3, 4] The results were, unfortunately, equivocal. Some slight darkening of the pigment occurred when the Zenker-fixed sections were "intensified" with sulfanilic acid and with ammoniacal silver. In fact, the darkening seemed as great as that observed in the medulla of control adrenal glands. In the latter, however, the zona reticularis of the cortex also became darker. Thick frozen sections, treated with phenylhydrazine, exactly as described by Bennett,[3] to demonstrate ketone bodies, took on a faint yellow color, considerably less than that of control adrenal glands. Similarly, some blackening, again less than that of controls, occurred with Reichstein's[3] silver solution. In frozen sections stained with Sudan IV, red-staining material was demonstrated only in a few cells, and these seemed to be in autolyzed portions of the tumor. Likewise, when the sections were examined in polarized light, a few tiny particles of anisotropic material, apparently identical in part with that which stained with Sudan IV, were seen after considerable search. In the medullae of control adrenal

glands, most of which had been fixed within 2 or 3 hours post-mortem, small amounts of Sudan-staining material were seen, but no anisotropic lipoids.

Several sections were stained by the ponceau-fuchsin method of Broster and Vines,[5] with several normal adrenals and cortical adenomas as controls. Some of the necrotic cells were stained red, but the cytoplasm of the better preserved cells took on a bluish tinge. The results were therefore regarded as negative. Iron hematoxylin stains (Goormaghtigh[6]) were negative, perhaps because of the rather extensive autolysis.

The *left adrenal gland* was found in its usual location. It was not remarkable in general shape, but was unusually thin, weighing only 3.6 gm. Section showed a narrow yellow cortex surrounding a gray, translucent medulla. Microscopically, both cortex and medulla were narrowed. The cortical cells were markedly vacuolated.

The *pituitary gland* measured 14 by 7 by 7 mm. and did not appear unusual in shape. It was fixed in Zenker's formaldehyde solution, embedded in paraffin and cut serially. Many sections were stained with hematoxylin and eosin, a modification of Mallory's acid-fuchsin aniline blue method,[7] and the Mann stain.[8] No adenoma was found. However, the majority of the basophile cells, which seemed to be somewhat reduced in number, showed to a striking degree the hyaline change described by Crooke.[9] Occasionally the basophile granules seemed to be completely replaced by the hyaline material (Fig. 10), but in many of the cells a small clump of granules remained, usually adjacent to the well preserved nucleus (Fig. 9). (For comparison, Fig. 8 is included, showing normal basophile cells, from the same case as Fig. 7.) The acidophile and chromophobe cells were well preserved and the posterior lobe did not appear remarkable.

Urinary Androgens

About 10 cc. of pale urine, recovered from the bladder post-mortem, was sent to Dr. Ralph Dorfman, who found, by a colorimetric method, a value of 130 mg. per liter for total 17-ketosteroids; by the same method the normal values for females range between 7 and 15 mg. per liter.

Assay of Neoplastic Tissue

About 100 gm. of the neoplastic tissue was cut into small pieces, preserved in 95 per cent alcohol and sent to Dr. Dorfman. Having previously failed to find androgens in adrenal tumors, he attempted to demonstrate cortin, but was unable to show any significant quantity, using the cold protection test in adrenalectomized rats.

A few grams of the neoplastic tissue which had been preserved in Klotz solution for several months was ground up and extracted with N/10 hydrochloric acid. When the extract was tested by the method of Folin, Cannon and Denis[10] a blue color appeared which was slightly more intense than that of a control solution of epinephrine (0.0001 per cent). However, the Vulpian, Comessatti, and Ewin's color reactions,[11] which were positive with the control solution, were negative when applied to the tumor extract. No attempt at assay on animals was made, since it was felt that the partially necrotic neoplastic tissue would certainly contain pressor substances of nonspecific type.

Post-Mortem Bacteriology

From the pleural fluid, the spleen, and urine *Staphylococcus aureus* and a nonhemolytic streptococcus were grown; from the heart's blood a nonhemolytic streptococcus and *Escherichia coli.*

Anatomical Diagnoses

A. Carcinoma(?) of adrenal gland (right); hirsutism; acne; atrophy of ovaries; hyaline change of Crooke in pituitary basophils; obesity, confined to head, neck and trunk; purple abdominal striae; cyanosis of head and neck; exophthalmos; arteriolosclerosis of kidneys; cardiac hypertrophy; osteoporosis; multiple healed fractures of ribs; calculus in left ureter.

B. Pulmonary emboli (source undetermined); pulmonary congestion and edema; pulmonary infarct in right lower lobe; fibrinous pleurisy (right); acute splenic tumor; cloudy swelling of viscera.

Discussion

The accurate histological identification of functioning adrenal tumors would seem to be of major importance in view of the far-reaching clinical conclusions which are being drawn from such cases. At the same time the number of such tumors which are apparently nonfunctioning is striking [12-14] (*cf.* Fig. 7).

A fairly well defined clinical picture of paroxysmal hypertension is being described with increasing frequency in association with pheochromocytoma, and epinephrine has been demonstrated in some of these tumors.[15-19b] It is interesting that hyperglycemia [19c] and "hypermetabolism" may be encountered in such cases and that the episodes of paroxysmal hypertension may resemble hypoglycemic shock.[18] In one of the cases reported by McCullagh and Engel,[18] urinary androgens were found to be within normal limits.

A syndrome of "adrenal virilism" associated with cortical hyper-

plasia or adenoma has been recognized for many years[16, 20, 21] although sometimes changes in the adrenals are not clearly demonstrated at operation or at necropsy.[22] More recently there have been increasingly frequent reports of hypertension (usually not paroxysmal) in association with hyperplasia or tumors of the adrenal cortex.[23, 24] It is both affirmed[25, 26] and denied[27] that the adrenal cortex is enlarged in cases of essential hypertension.

In Cushing's syndrome the situation is even more complex. As Haymaker and Anderson[20] have pointed out, we have passed through a "pituitary epoch" in the study of this disease and are now in the midst of an "adrenal epoch," the general tendency being to interpret the syndrome as a manifestation of hyperfunction of the adrenal cortex.[16, 28–30] Yet the only constant pathological finding seems to be the hyaline change of Crooke[9, 31, 32] in the pituitary basophils, which would perhaps suggest that the fundamental disorder, whether "primary," or "secondary" to something else, may lie in the anterior pituitary gland and that the name "pituitary basophilism" may properly be retained (as Crooke and Callow[33] have done) for all these cases.

Since Cushing[34–36] described the syndrome, a good many cases have been recorded exhibiting a similar group of symptoms. The patient herein described presented practically all of the signs and symptoms which have been mentioned in reported cases of this disease in adult women; *viz.*, amenorrhea, hypertension, hyperglycemia difficult to control with insulin,[37] polyphagia,[38] obesity confined to head, neck and trunk, the typical "moon face" with cyanosis of head and neck, purple abdominal striae, hirsutism, acne, osteoporosis with multiple fractures,[39] renal calculi, feelings of fatigue or apathy, and mental symptoms.[40] The high urinary androgen excretion and progressive enlargement of the clitoris have been noted particularly in the cases associated with tumors diagnosed as carcinoma of the adrenal cortex.[33, 41] Death from overwhelming infection is also common. (In this instance the axillary abscess may have given rise to both the septicemia and the pulmonary emboli.) Indeed, the only frequently described clinical finding which was absent in the case here reported was polycythemia. From the anatomical point of view, renal arteriolosclerosis[42, 43] is mentioned fairly frequently and the hyaline change of Crooke[9] in the pituitary basophils, when looked for, appears to be a constant finding.

Since the Cushing syndrome is the subject of much recent and current discussion,[16, 20, 21, 28, 29, 33, 38, 41, 44–47] no attempt will be made to review the literature in detail. However, in reading summaries of reported cases, one is struck by what seems to be an outstanding dis-

crepancy; namely, the relative constancy of the clinical symptoms and the variability of the pathological findings (with the exception of Crooke's change in the pituitary gland). The syndrome has been reported in association with basophile adenoma of the pituitary body, with tumors of the thymus, with carcinoma, adenoma, or hyperplasia of the adrenal cortex, with arrhenoblastoma(?),[48] and finally, without any of these findings.[49] Almost as remarkable, perhaps, is the apparent fact that any of these tumors or hyperplasias may occur in the absence of the clinical symptom complex.

The tumor in the present case was at first regarded as a carcinoma of the adrenal cortex, and some support seemed to be given this hypothesis by the atrophy of the other adrenal gland. Further study, however, and especially the finding of pigment in some of the cells in the Zenker-fixed tissue but not in formaldehyde-fixed material, cast doubt on the diagnosis. As already noted, histochemical studies were not conclusive, and Dr. Ralph Dorfman was unable to demonstrate significant amounts of cortin in the neoplastic tissue. Therefore the question arose whether the tumor might not be a pheochromocytoma. The likelihood of simultaneous origin from both cortex and medulla seemed remote.

A section of the tumor was shown to Dr. Fred Stewart who felt at first that its appearance and the presence of pigment in the Zenker-fixed tissue suggested a medullary origin. After further study he said that he thought it impossible to be certain that it was not a cortical tumor. A slide was also sent to Dr. Howard T. Karsner, who stated that on an objective basis he would favor a diagnosis of pheochromocytoma, while admitting that because of its cellular character it could conceivably have arisen from the zona reticularis. Dr. Karsner also remarked that he had rarely, if ever, seen a "pure" medullary tumor of the adrenal; *i.e.*, that most of these tumors have components resembling cortical tissue. This he does not take to mean necessarily that the tumors are of mixed cortical and medullary origin, but rather that neoplastic medullary cells may come to resemble cortical cells morphologically.[50]

Through the courtesy of Dr. Louise Eisenhardt, I had the opportunity of examining slides from four reported cases of adrenal tumors associated with Cushing's syndrome. Two were similar to the present case, showing sheets of cells, most of them with abundant, acidophilic, granular, slightly vacuolated cytoplasm, separated by large sinusoidal blood vessels, but without definite pigmentation. (It was not known whether these tissues were fixed in chromate solutions.) Another showed a cord-like arrangement of "foamy" cells with numerous mitotic figures. Still another contained markedly vacuolated cells,

reproducing, rather faithfully, layers of the adrenal cortex, like the adenoma shown in Figure 7. These slides were also shown to Dr. Stewart, who thought that two of them could possibly be of medullary origin.

Thus it is evident that the histological diagnosis in the case here reported, and perhaps in some others as well, was a matter of considerable difficulty. In this connection some discrepancies in the literature may be noted also. For example, in the case of Porter and Porter,[51] an adrenal tumor, removed in a case of paroxysmal hypertension with, apparently, no other outstanding symptoms, was called a cortical adenocarcinoma by Ewing, who also stated that it contained no pigment. No statement was made regarding fixation in chrome salts. In describing a case of chromaffin cell tumor associated with paroxysmal hypertension, Coller, Field and Durant[52] stated that such tumors may so closely resemble cortical tumors that the diagnosis must be questioned if chromate fixation is not used. Cragg[53] cast doubt on the relation of the chromaffin substance to epinephrine. The situation is hardly clarified by the statement of Geschickter,[54] in describing benign cortical tumors, that "deposits of chromaffin (*sic*) pigment are common."

Of particular interest is the case recently reported by Neff, Tice, Walker and Ockerblad[55] of an adrenal tumor in a female infant in whom symptoms began at 8 months with onset of hirsutism, acne, hypertrophy of the genitalia, and, to judge from their illustrations, a puffy obesity of the face. The child had hypertension which is said to have been paroxysmal. Amelioration of symptoms occurred following removal of the tumor, which contained vacuolated, fat-containing cells and "brown granules were seen in many of the cells when the tissue was treated with chrome salts; silver impregnation permitted a diagnosis of chromaffinoma or pheochromocytoma." Although this case cannot be regarded as showing a full-blown Cushing's syndrome, the histology of the tumor seems remarkable when the symptoms are considered. The authors' suggestion that the tumor caused dysfunction of the cortical cells by pressure is not convincing.

Some of the adrenal tumors encountered in cases of Cushing's syndrome have been said to reproduce the structure of the zona reticularis of the normal adrenal, and such an appearance has been linked with the hypothetical "androgenic zone" of the fetal adrenal. The chief proponents of this point of view in the past have been Grollman[56] and Broster.[5, 57, 58] More recently Gersh and Grollman[59] seem to have abandoned Grollman's original position concerning the production of androgens by the adrenal cortex and, in fact, go so far as to suggest that

the androgenic effects seen in pathological conditions may be due to misplaced testicular tissue in the adrenal. Others have questioned the androgenic function of the so-called "x-zone."[60] Related to the concept of the androgenic zone is the use of the ponceau-fuchsin stain of Broster and Vines.[5] This is supposed to stain the cytoplasm of the androgenic cells a brilliant red. As previously noted, this stain was negative in the present case. The failure to secure a positive fuchsinophile reaction in my case may have been due to post-mortem change in the tissue. However, the specificity of the stain seems open to serious question.[3,12,14,49,50,61,62] The siderophile granules described by Goormaghtigh[6] in masculinizing adrenal tumors were also looked for in the present case and were not found, again possibly because of post-mortem autolysis.

The presence of lipoids, or at least of vacuolation in the tumor cells, is commonly mentioned in the literature on cortical tumors, and it is stated by Cahill, Melicow and Darby[12] that the tumors in their series which were associated clinically with symptoms suggestive of Cushing's syndrome contained more vacuoles than the others. In my case, frozen sections stained with Sudan IV showed positive staining only in areas which appeared to be necrotic. Frozen sections were also examined in polarized light, both with and without treatment with digitonin,[3] and negligible amounts of anisotropic lipoids were seen. In this connection, it should be remarked that fat vacuoles have been mentioned, though rarely, in reported cases of pheochromocytoma,[55] as well as in tumors of the carotid body[63] (*cf.* also the statement of Karsner,[50] cited previously). Also, it must be remarked that wide fluctuations, including extreme depletion, may occur in the amount of fat in the cortex of normal adrenal glands under various physiological and pathological conditions.[26,64,65] It is reasonable to suppose that the fat content of tumors may vary in a similar way.

The presence of brown pigment in the cells after chromate fixation has perhaps been given greatest weight as a differential criterion between cortical and medullary tumors. However, even this may be inconclusive, since the zona reticularis of the normal adrenal cortex contains yellow-brown pigment granules, of uncertain nature, and these may appear in cortical adenomas. Conceivably, of course, the distribution of pigment in a tumor may be so uneven that it may appear in some sections and not in others.

In regard to the general cyto-architecture of adrenal tumors, it is generally stated that the cells of cortical tumors are likely to be arranged in cords, imitating more or less the normal cortex, while in the pheochromocytoma the cells are usually said to be arranged in sheets

or nests, to be polyhedral, with rather abundant cytoplasm, and to vary considerably in size and shape, with occasional giant forms.[12, 15, 19b, 66, 67] But Wu,[68] in a review of carcinoma of the adrenal cortex, emphasized the frequency of "pleomorphism" of cells.

It is usually said that cortical tumors are rarely malignant, and pheochromocytomas apparently metastasize only in exceptional cases. The tumor in the case here reported was not definitely malignant, although there was a suggestion of an invasive tendency in the nodule protruding beneath the endothelium of the suprarenal vein.

Additional evidence of the complexity of the situation with regard to functioning adrenal tumors is the apparent fact that the cortical origin of all of the so-called cortical hormones, while extremely likely, is not established beyond all question. In the recent review of the cortical hormones by Reichstein and Shoppee[69] the following statement appears: "In recent years whole glands rather than the dissected cortices have been used almost exclusively as starting material; this is also the case for the industrial preparation of the extracts used in clinical work. Nevertheless, it is highly probable that the steroids originate from the cortex and not from the medulla." (Reference is then made to the histochemical work of Bennett, cited previously.)

Kendall[70] stated that, although he has never tried to recover cortical hormones from a pheochromocytoma, he is "quite certain" that cortical hormones are not produced by medullary tissue.

Some of the differential histological criteria may be summarized as follows: in favor of a cortical tumor are (1) cord-like arrangement of cells, (2) fat vacuoles in cells, (3) absence of brown pigment after fixation in chromate solutions or other oxidizing agents.* In favor of a medullary tumor, on the other hand, there may be considered: (1) polyhedral cells, varying in size and shape, arranged in sheets or nests; (2) absence of fat in tumor cells; (3) presence of pigment after chromate fixation. It is obvious, however, that in some cases, such as the one here reported, the application of these criteria may not result in a clear-cut answer (*cf.* also Coller, Field and Durant[52]).

Finally, in view of the evident difficulties of deciding in some cases whether an adrenal tumor is of cortical or medullary origin, and the general uncertainty regarding the functions of such tumors, it may be suggested that future cases, especially when associated with Cushing's syndrome, be carefully studied by histochemical methods and by prompt assay of the fresh neoplastic tissue for both epinephrine and

* Bennett[4] pointed out that the term "chromaffin" is a misnomer, since the brown pigment is not a chromium compound, but the product of oxidation of epinephrine or related substances and can be obtained by the use of other oxidizing agents.

steroid hormones. Information gained in this way should be of more value than that derived from simple inspection of paraffin sections, and should permit better correlation with the clinical findings.

Summary

A case of Cushing's syndrome associated with an adrenal tumor of uncertain histogenesis, possibly a pheochromocytoma, is described. A high content of 17-ketosteroids was found in a single sample of postmortem urine, but no significant amount of cortin was demonstrated in the neoplastic tissue.

Application of the usual histological criteria as well as more refined technics did not yield a clear-cut answer as to the origin of this neoplasm. Careful study by histochemical methods and by assay of fresh tissue for epinephrine and steroid hormones may be expected to give more definite information than routine histological methods in the investigation of adrenal tumors associated with Cushing's syndrome.

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DESCRIPTION OF PLATES

PLATE 135

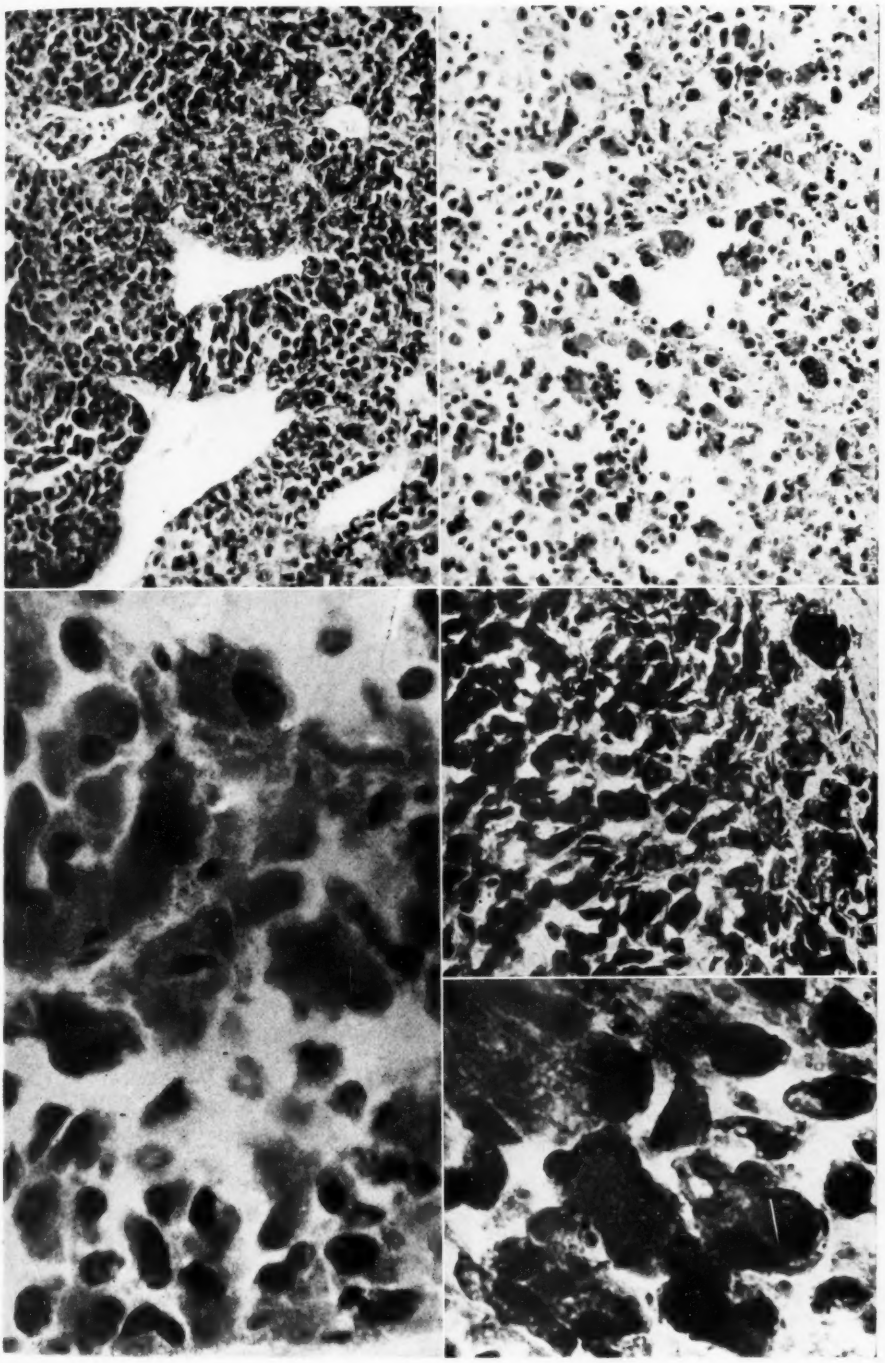
FIG. 1. A part of the adrenal tumor in which the cells are relatively small and arranged in compact sheets surrounding large sinusoids. Hematoxylin and eosin stain. × 200.

FIG. 2. Another portion of the tumor, showing a quite different structure. The size of the cells may be compared with those in Figure 1, which is taken at the same magnification. Hematoxylin and eosin stain. × 200.

FIG. 3. Higher magnification of cells from a field similar to that in Figure 2, to show variation in size and shape. One or two cells appear to have small vacuoles in the cytoplasm. Hematoxylin and eosin stain. × 700.

FIG. 4. Another field, showing hyperchromatic nuclei and deeply staining cytoplasm. A tumor giant cell is seen in one corner. Hematoxylin and eosin stain. × 350.

FIG. 5. A group of pigmented cells adjacent to a sinusoid. In the original preparation the cytoplasm of these cells has a diffuse yellow-brown color. Hematoxylin and eosin stain. × 750.



LeCompte Cushing's Syndrome and Pheochromocytoma

PLATE 136

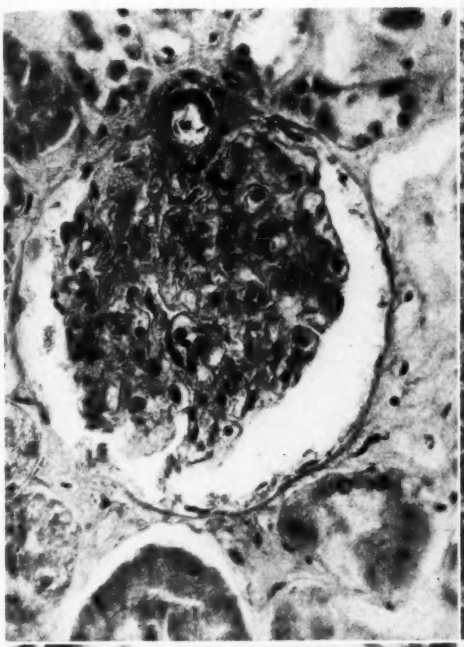
FIG. 6. Hyaline thickening of afferent arteriole in kidney. Hematoxylin and eosin stain. × 335.

FIG. 7. Representative field from an apparently nonfunctioning cortical adenoma about 3 cm. in diameter, encountered unexpectedly at autopsy in an elderly woman. This is for comparison with sections of the tumor in Plate 135. Hematoxylin and eosin stain. × 125.

FIG. 8. Normal basophile cells, from the same case as Figure 7, for comparison with Figures 9 and 10. The cell near the center with large, eccentric nucleus is an eosinophil. Mann stain. × 1600.

FIG. 9. Two basophile cells from the pituitary gland of the case reported, to show hyaline change of Crooke. The granules are partly replaced by hyaline material. Mann stain. × 1600.

FIG. 10. Two more basophile cells, one a very large one, from the pituitary gland of the case reported. The granules have been almost completely replaced by hyaline material. For comparison with Figure 8. Mann stain. × 1600.



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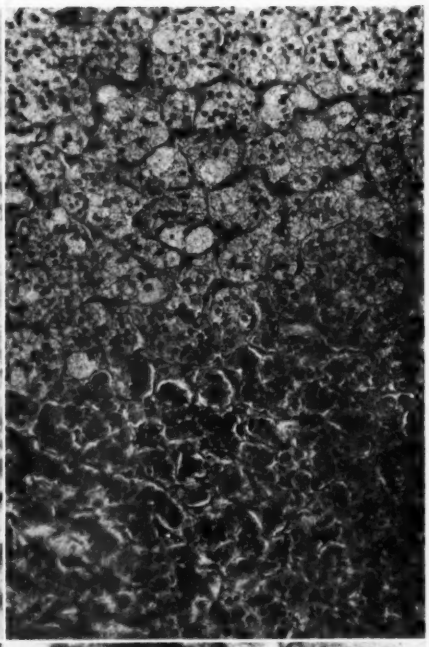

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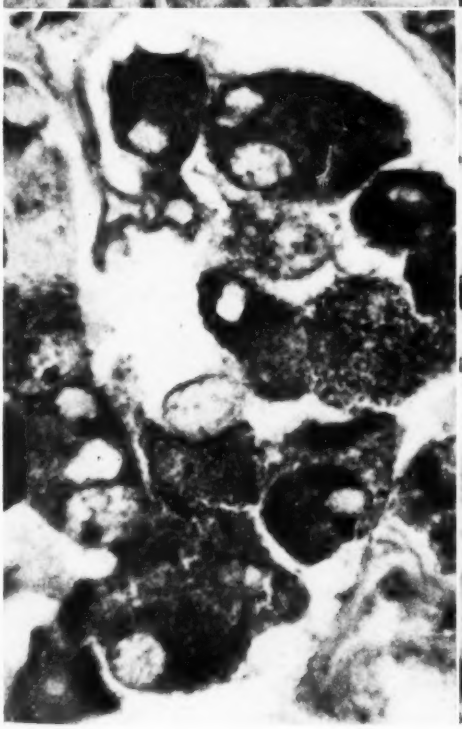

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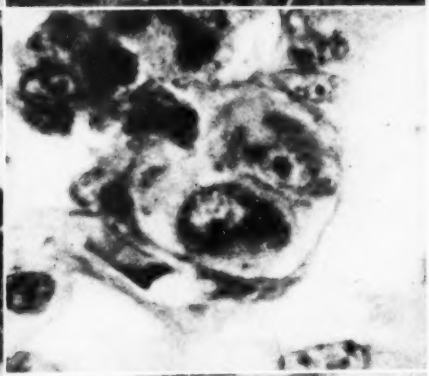

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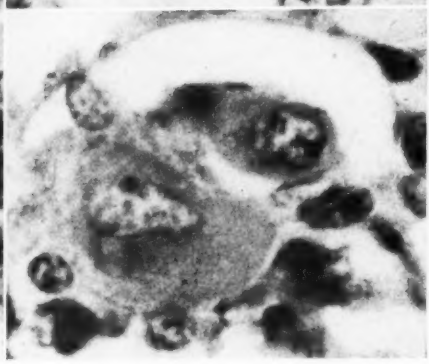

10

KERATODERMIA BLENNORRHAGICUM

Report of Case with Autopsy *

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Although gonorrhea is still one of the most common infectious diseases, the general systemic form, keratodermia blennorrhagicum or gonorrheal keratosis, is one of the very rare causes of death. While the disease is known to be a general systemic one, with the most striking local manifestations in skin and joints, most studies have been limited to biopsies of the skin and clinical observations. Only a small amount of experimental work has been done and, because the disease is seldom fatal, complete autopsy reports and anatomical studies are singularly deficient in the literature.

A review of the literature reveals reports of only five fatal cases of gonorrheal keratosis, and of these only three include the results of autopsy. The fatal case of Lees and Percival,[1] and one of the two fatal cases of Rost[2] were apparently not studied by necropsy. The other case reported by Rost is imperfect because this patient was never known to have had gonorrhea. However, a typical history of arthritis, preceding by 1 year a pustular and later hyperkeratotic lesion of the skin, makes the clinical diagnosis credible.

In the three cases corroborated by anatomical studies, most of the essentials have been found. In Rost's[2] second case the post-mortem examination showed a small heart, generalized muscle atrophy, slight thickening of the tricuspid valve and early arteriosclerosis, particularly marked in the aorta and kidneys. The testicles were small, but otherwise normal in appearance, and there was a generalized bony ankylosis. Simpson[3] found in his case localized fibrinous pericarditis and some fibrous adhesions from the spleen to the diaphragm. There was chronic prostatitis, seminal vesiculitis, testicular atrophy together with a generalized deforming polyarthritis and passive congestion of the viscera. Simpson made bacterial studies of the prostate, seminal vesicles and joint fluid, but found no gonococci. Scholtz[4] found involvement of the serous membranes, but no fibrinous exudate on serous surfaces. There were general serous effusions, however, the largest being 150 cc. of fluid in the pericardial cavity. He found also early bronchopneumonia, an hyperplastic spleen and a very active bone marrow. The synovial surfaces of the various joints were examined in his case and, although

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there was a generalized partial ankylosis, the synovial surfaces were smooth and showed no adhesions or fibrinoid deposits. The ankylosis was in a position of semiflexion. No fluid was found in any of the joints. The skin lesions in this report are designated as extremely numerous and generalized.

All of these cases with fatal termination have been those of males who have had an unusually persistent, resistant and generalized arthritis with deformities, and they have all shown generalized skin lesions. Excepting the case reported by Simpson,[3] the disease has terminated with a bronchopneumonia and marked emaciation. These cases, in common with nonfatal cases, pass usually through a stage of fever, toxemia and debility, but among the nonfatal cases reported only 2.7 per cent have had generalized skin lesions. Because of the rarely fatal termination of this form of gonorrhea, complicated by typical skin changes and a generalized ankylosing arthritis, we wish to report a case of keratodermia blennorrhagicum with autopsy.

Report of Case

R. P. H., a white, male laborer, first developed typical acute gonorrheal urethritis in 1929, at the age of 20. Three weeks later he began to show the signs and symptoms of acute arthritis of one knee, with swelling, heat, tenderness and restricted motion. The inflammatory process appeared serially in all of the larger joints of the body, and the patient was hospitalized for 8 months, during which time there was continual involvement of at least one or two joints. He was then discharged but continued to have moderate symptoms for 2 years and was not completely well until after an appendectomy was performed in 1932. From 1932 until 1940, the patient was symptomatically well.

In March of 1940, at the age of 31, there was a recurrence of acute arthritis which again involved every joint which had been involved previously, and also some of the smaller joints. He entered the San Francisco Hospital on June 12, 1940, at which time the joints involved were the left sternoclavicular, the left fourth metacarpophalangeal, the right third metacarpophalangeal, several metatarsal joints in both feet, and the right elbow. He was given a course of six pyrotherapy treatments, with considerable improvement, and was discharged to the Clinic service. At this time he was having a morning urethral discharge of a few drops, but several examinations failed to show gonococci. There was also atrophy of the right testicle, and enlargement of the inguinal lymph nodes. A cutaneous Frei test was negative. The skin was dry and scaly, with an acne-like eruption upon the trunk, and at this time a diagnosis of seborrheic dermatitis was made.

The patient then went to Des Moines, Iowa, believing that the warmer climate would help his arthritis. Instead he had a relapse. He was in the Des Moines Army Hospital for 5 months, during which time he received a "complete course" of sulfanilamide and sulfathiazole.

Aside from a small patch of dry, scaly skin upon the scalp for 2 years previously, the major skin lesion appeared while the patient was hospitalized in Iowa. It was described as a nonpruritic rash, believed to have started as patches upon the extensor surfaces of knees, elbows and shoulders, but spreading over the entire body. The lesion appeared before sulfa drugs were given, and there is no history of medication with bromides, barbiturates, iodides, or other drugs capable of producing dermatitis of this type.

He re-entered the San Francisco Hospital on December 23, 1941, with an acute relapse of arthritis and persistent dermatitis.

Physical Examination. Examination at this time showed an acute arthritis of the ankles, with a less acute involvement of the knees (which were still tender), moderate involvement of the elbow and shoulder joints, and some deformities of the metacarpophalangeal joints in the hands. There was generalized muscle atrophy and cachexia. There were several carious teeth. The blood pressure was 110/70, and the remainder of the general examination revealed nothing abnormal.

The skin showed a generalized papular eruption consisting of discrete but often coalescent, ragged, crusted lesions. On the trunk they were rupial and resembled shallow cones, with a grayish tan or brown color. They ranged from 3 to 15 mm. in diameter, and were so numerous that in most places they were almost contiguous. On the extremities, especially on the plantar and palmar surfaces, the lesions were often coalescent to form large, irregular, wandering, elevated patches of cornified, crusted material. Lesions on the dorsum of the hands and on the scalp were considerably smaller but just as numerous and were pale, yellowish gray and of a smoother contour than the larger ones seen elsewhere. A few of them resembled "droplets of wax." These lesions were the more easily removed and beneath them the skin was moist, slightly reddened, and level with the surrounding skin. The lesions were most numerous upon the trunk and tended to avoid the body folds and also the areas of skin in contact with the bed. In the groins and axillae, and to a lesser degree in the other body folds, the skin was moist and reddened and showed numerous white areas which were slightly elevated and somewhat macerated. The nails were thickened, white and scaling. There were decubital ulcers on the heels and over the sacrum and midportion of the back.

Laboratory Findings. The hemoglobin was 65 per cent, the red blood cell count was 2,800,000 and the white blood cell count 9,200 per cmm., with a normal smear and differential count. Urine and stool were normal. Wassermann and Kahn tests of the blood were negative.

Therapy. The patient was treated with potassium permanganate baths, vitamin A and cod liver oil. The arthritis continued unabated, and the temperature ranged from 100° to 102° F. The patient was in extreme distress almost continually and required increasing doses of morphine. He gradually became cachectic, had a persistent toxemia and finally developed bronchopneumonia and expired. The skin lesions had failed to respond to any type of local therapy.

Necropsy

The autopsy on January 8, 1942, showed an extreme degree of emaciation and generalized, partial ankylosis with restricted mobility of the joints, with the more noticeable deformity in the hands and wrists. There was marked edema and some reddening of the ankles, which were still warm at the time of autopsy. The skin showed essentially the lesions described clinically.

Upon opening the body the peritoneal and pericardial cavities were without adhesions or exudate, while the pleural cavities showed only a few old fibrous adhesions. There were no effusions.

The lungs were edematous and showed areas of consolidation about the bronchi with numerous focal abscesses in each lower lobe.

The prostate weighed about 30 gm., was homogeneous in consistency, and a little more dense than normal. The right testicle was rather

small, but showed no inflammation or fibrosis. The aorta showed a few small atheromatous plaques in its abdominal portion, but no evidence of lues. In the right ankle joint small fibrous adhesions crossed the joint cavity in several places, with thickening and fibrosis of the synovial membrane, while other synovial membranes were clean and smooth. There was no effusion into this, of other joints, although there was edema around the right ankle. The heart, liver, spleen, pancreas, stomach and intestines, kidneys, ureters, bladder, adrenals, thyroid gland, pituitary body and brain showed no gross pathological changes.

Microscopic Examination. Sections of the skin lesions showed all of the changes described in detail by Keim[5] and re-emphasized by numerous writers, except for vesiculation.

The earliest change has not been a constant finding of the various writers and might not be expected when the disease was sufficiently advanced to cause death. The earliest lesion (Fig. 1) is a tiny collection of polymorphonuclear leukocytes in the stratum granulosum of the skin with elevation of the stratum lucidum to form a little nest. The prickle cell layer is not involved at this time. As the process advances, the corium begins to show infiltration of lymphocytes and some edema of the dermal papillae. The lymphocytic invasion is restricted to the superficial layer of corium and is rarely found deeper or in the subcutaneous tissue. It persists throughout the life of the lesion. A few plasma cells also may be found, but in the progressing lesions only rarely are there any polymorphonuclear leukocytes. Later the superficial pustules gradually become larger, further elevating the keratinized layer, and soon produce a noticeable depression of the prickle cell layer, but the lesion is still very well circumscribed (Fig. 2). This pustule now shows various degrees of keratinization, forming a smooth, rather opaque, pale-staining mass which fuses with, and becomes indistinguishable from, the old stratum lucidum. Meanwhile a new stratum lucidum has begun to form beneath this keratinizing mass of polymorphonuclear cells, and can be seen extending in from the edges toward the center (Fig. 3). This new stratum lucidum is obviously formed by keratinization of the superficial layer of prickle cells in contact with the pustular mass. It soon becomes complete, forming a dimple in the intact skin with the superficial lesions still forming a conical elevation at the same spot. The keratinizing process is now complete; no new cells infiltrate the lesion, and the mass soon becomes almost homogeneous except for a few multilobed nuclei scattered through it. The underlying skin at this point gradually flattens out so that the keratinized mass is quite definitely elevated, and the under-

lying skin is level with the surrounding normal skin. The skin is now intact, with lymphocytes in the corium and with a large squamous crust upon the surface. At this point the crust may desquamate, leaving unscarred, normal skin (Fig. 4), or a new lesion, developing at this time or just prior to it, may be ripening and may produce another layer of crusting. This repetitious process eventually produces the characteristic large, irregular, horny lesions seen upon the extremities.

The exemplary lesion just described was the typical one upon the scalp, where the lesions were fairly smooth and discrete and did not show brownish coloration. Acanthosis was either very slight or only moderate in amount, never exceeding the degree seen in Figure 5. Parakeratosis was observed to a very limited degree in the early lesion. It is possible that it is only simulated by separation of layers of keratinizing material, without an actual absolute increase in the amount of the stratum lucidum. The prickle cell layer, basal layer, and subcutaneous tissue showed no evidence of involvement.

Occasionally a mild endarteritis of medium and small-sized arteries in the subcutaneous tissue was found. These arteries showed swelling and irregularity of the endothelium, and slight subintimal fibrosis. There was no other evidence of reaction at this level.

Bacterial stains of these sections have failed to demonstrate organisms in either the lesions or the surrounding skin.

Section of tarsal joints in the right ankle showed a few small adhesions bridging the synovial membranes, with considerable fibrosis around them and with several dense cordons of lymphocytes and plasma cells in the adjacent collagen (Fig. 6). There was a mild chronic prostatitis and a subacute posterior urethritis. No bacteria were found in these fixed tissues. Sections of skeletal muscle showed diminution in diameter of the muscle fibers and an apparent or relative increase in the number of nuclei of the sarcolemma, the capillaries and the endothelial cells (Fig. 7). No inflammatory infiltration was seen. There was an unusually large number of macrophages, filled with yellowish brown pigment giving a positive reaction for iron, in the lymph nodes of the hilar and abdominal region, in the gastric mucosa, the bone marrow, spleen, liver, the inflamed portion of the ankle joints and in the posterior lobe of the pituitary gland. A moderate amount of iron pigment was seen in the spleen. In the liver there was considerable fatty infiltration and mild congestion. There was a well developed, confluent bronchopneumonia, and in the right testicle the seminiferous tubules showed atrophy and were separated by diffuse fibrosis. The bone marrow was active. Sections of the other organs were normal. Unfortunately, culture of the blood was not done.

Discussion

The history and skin lesions in this case are consistent with keratodermia blennorrhagicum, although the skin lesions persisted over a long period. Since the original arthritis preceded the dermatitis by about 10 years, we may consider psoriasis arthropathica to be extremely unlikely. Of the other criteria set up by Epstein[6] for the differential diagnosis of these two diseases, almost every one points toward gonorrheal keratosis.

The course of this case was similar to that of the other fatal cases, with a generalized eruption, marked emaciation, exhaustion, persistent fever and, finally, bronchopneumonia. The ankylosis was not so well developed as in some of the reported cases, and the arthritis was less active. Prostatitis, urethritis and testicular atrophy are all part of the inflammatory process of the genital tract, and are familiar observations in this disease. Testicular atrophy is the most characteristic of these changes. The other findings were all consistent with a state of starvation and chronic toxemia. The distribution of pigment may indicate a general, slow hemolysis, accompanying toxemia and producing anemia. The absence of bacteria in the tissues is in accord with the majority of previous reports.

Although generalized dermatitis indicates a poor prognosis in keratodermia blennorrhagicum, we still wish to emphasize two facts in regard to therapy. One is the failure of sulfanilamide and sulfathiazole to alter appreciably the course of the disease, although administered in large doses during an arthrocutaneous relapse, to a person not previously treated with these drugs. The other point is the appearance of skin lesions following a series of pyrotherapy treatments (to 104° F.) for the arthritis, in spite of the strong recommendation[7] of pyrotherapy as a treatment for the dermatitis itself.

Summary

Necropsy of a patient with keratodermia blennorrhagicum showed generalized chronic arthritis with a few adhesions in the joints, typical lesions of the skin in varying stages which permitted study of their evolution, chronic prostatitis, chronic posterior urethritis, testicular atrophy, fatty infiltration of the liver, and cachexia. Bacteria could not be demonstrated in sections of the skin. There were no new findings which might help in explaining the cause of this disease.

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[*Illustrations follow*]

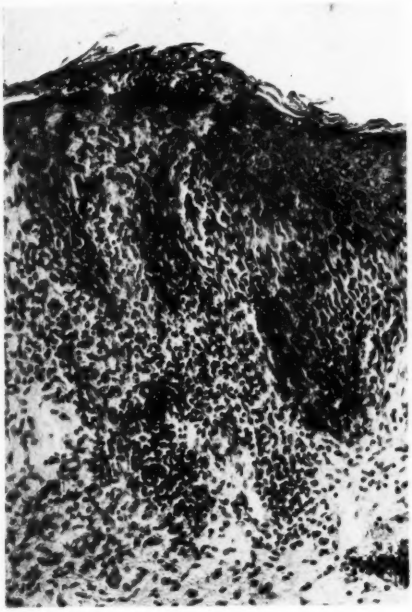
DESCRIPTION OF PLATES

PLATE 137

FIG. 1. Earliest lesion seen, with pustule beneath the stratum corneum and lymphocytes in corium. × 145.

FIG. 2. Larger pustule than that shown in Figure 1, with cellular débris fusing and hyalinizing. × 95.

FIG. 3. Pustule a little older than that shown in Figure 2, with new corneal layer separating the older exudate and cellular débris from the prickle cell layer. × 120.



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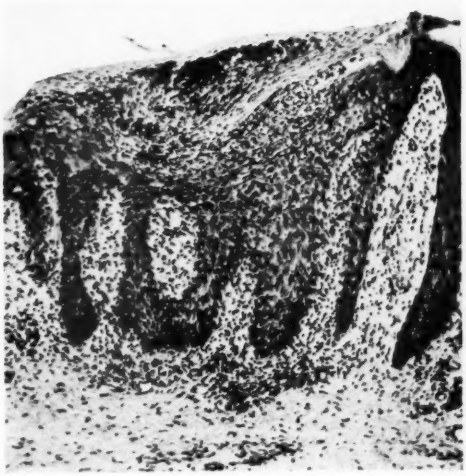

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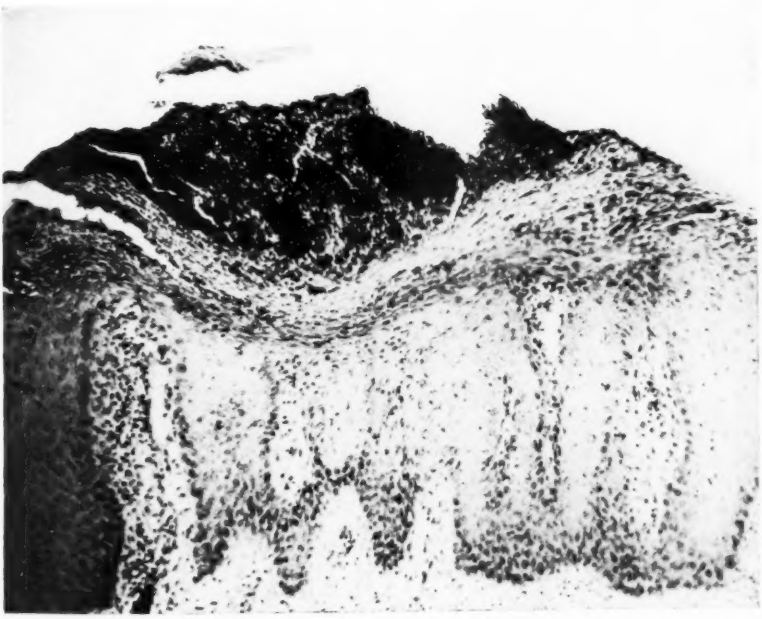

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PLATE 138

FIG. 4. Former pustule, now a squamate crust, separating from the intact skin beneath, which is now level with the surrounding skin. There is an older pustule on the right, with moderate squamate transition, which is being elevated by the newer pustule on the left. Parakeratosis is present in this area. × 90.

FIG. 5. Early pustule, showing greatest degree of acanthosis seen. No parakeratosis is evident. × 93.

FIG. 6. Left ankle joint with active synovitis, adhesions and lymphocytic infiltration. × 140.

FIG. 7. Psoas muscle. Proliferation of endothelial and sarcolemmic cells. × 140.

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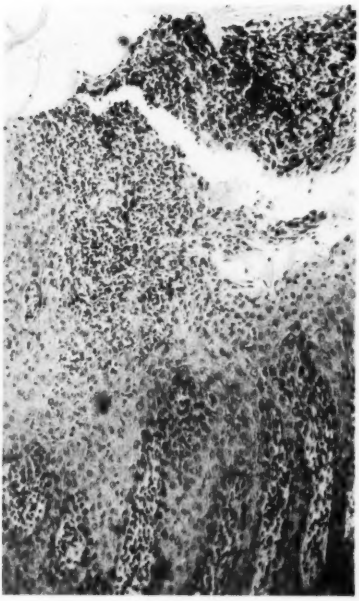

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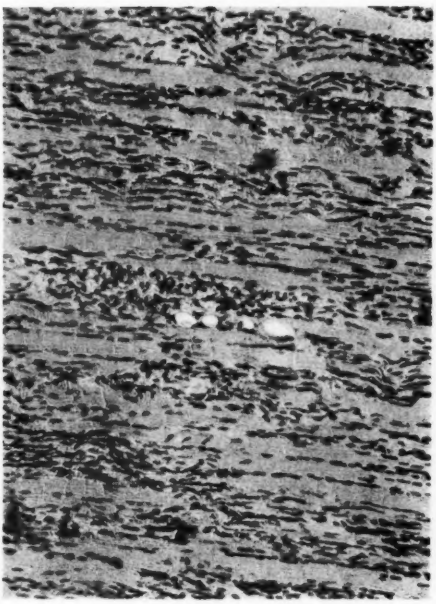

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Keratodermia Blennorrhagicum

PERIARTERITIS NODOSA IN EXPERIMENTAL HYPERTENSIVE RATS AND DOGS*

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In the course of a procedure to test the effect of certain renal extracts upon experimentally produced hypertension in rats and dogs, it was discovered at autopsy that many of the animals had periarteritis nodosa. The lesions found in these animals appeared to be identical in gross and microscopic morphology and in distribution with those of human periarteritis nodosa. A study of this condition in these animals was begun with the hope that it would add to our knowledge of the occurrence of similar lesions in man.

LITERATURE

A summary of the reported occurrence of periarteritis nodosa in animals is presented in Table I, inspection of which reveals that the disease has been found in a wide variety of animals, including cows, pigs, deer, dogs, rabbits and rats. Spontaneous occurrence of isolated cases in animals has been recognized for many years. Wilens and Sproul found many cases in very old rats fed a special diet.

Periarteritis nodosa has been described recently in rats in which hypertension was produced either by placing a silver clamp on one renal artery (Wilson and Byrom), or by wrapping one kidney with cellophane (Friedman, Jarman and Klemperer), or by various other operative procedures to induce hypertension (Cromartie).

Only 3 cases of periarteritis have been described in hypertensive dogs. Child reported its occurrence in 1 hypertensive dog following production of an Eck fistula. Goldblatt † states that he has found the disease in only 2 of more than 1000 dogs examined, while Graef and Page did not mention its occurrence following the production of hypertension by cellophane perinephritis.

No reports have been found of the occurrence of periarteritis nodosa in animals in which the blood pressures were proved to be normal, or in which the kidneys were proved to be free from pathologic change.

METHODS

Clinical and pathological studies were made on 100 rats and 8 dogs used for routine assays of the blood-pressure-lowering activity of cer-

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† Personnal communication.

TABLE I

Reported Occurrence of Periarteritis Nodosa in Animals

Animal	Author	Year	Arteries involved	Remarks
Calf	Guldner	1915	Heart, skeletal muscles	Spontaneous occurrence in a 4-weeks-old animal
Cow	Nieberle	1928	Heart, kidney, liver, spleen, lymph nodes, udder, gall-bladder	Spontaneous occurrence in a 3½-year-old animal
Deer	Lüpke and Jaeger	1906 and 1909	Abdomen, heart, vasa vasorum	This disease appeared in a herd of 100 10-year-old axis deer, born and living in a park in Ludwigsburg; the etiologic factor was thought to be bacterial but attempts to isolate organisms were unsuccessful; the disease was not found in other similar herds in Calcutta and Hamburg.
Dogs	Baló	1924	Heart	Spontaneous occurrence in a 4-year-old male fox terrier, which had acute gastritis, parenchymatous nephritis and chronic mitral endocarditis
	Child	1938	Widespread, especially in heart and mesentery	One of 12 dogs with experimental hypertension produced by constricting renal artery; in this animal an Eck fistula, produced after hypertension was maintained for 6 months, was followed by a slow rise to 300 mm. Hg, ending in hemiparesis and death
	Goldblatt	1941*	"Most manifest in the heart"	Occurrence in 2 of more than 1000 dogs examined; both were hypertensive with uremia
Pigs	Joest and Harzer	1921	Heart, kidney	Spontaneous occurrence in a 12-month-old animal which was lame and had pseudomembranous inflammation in the mouth and throat
	Collins and Goldie	1940	Heart, kidneys, mesentery, synovial membranes	Occurred in 5 of a group of animals given *Erysipelothrix rhusiopathiae* to produce arthritis; absent in controls and in those injected with streptococci
Rabbits	Harris and Friedrichs	1922	Lung, liver, heart, kidneys	Authors made an emulsion from kidneys of a typical human case of periarteritis nodosa, injected it into ear veins of rabbits which then developed suggestive lesions; emulsions of their involved organs were passed through Berkefeld filter and the filtrate injected into the ear vein of another rabbit which subsequently presented "typical lesions"

Rabbits				were passed through Berkefeld filter and the filtrate injected into the ear vein of another rabbit which subsequently presented "typical lesions"
	Wilson and Pickering	1938	Intestines, stomach, suprarenal gland, liver, heart, eye	Experimental hypertension produced by constricting renal arteries with silver clamps; lesions described as periarteritis nodosa, but not so designated; lesions seemed to be related to rapidly developing severe hypertension
	Rich and Gregory	1943	Widespread	Lesions occurred in 12 of 14 rabbits in which hypersensitivity to horse serum was induced experimentally; controls were not described; 10 of the 14 rabbits had acute diffuse glomerulonephritis; blood pressure studies were not reported
Rats	Metz	1931	Kidneys, lungs, heart, brain, liver, skin, skeletal muscle	Lesions occurred in white rats following injections of *Streptococcus hemolyticus*, sterile toxin and nonspecific protein antigen, to which, respectively, the animals had been highly sensitized
	Masugi	1935	Kidneys, intestines, lungs, heart, aorta	Lesions occurred in rats which had been injected with rabbit anti-rat-kidney serum.
	ilens and Sproul	1938	Widespread	Lesions found in 47, or 9.7%, of a large series of rats studied for cardiovascular disease; these animals were old rats used in a dietary experiment, in which the diet consisted of dried milk and ground wheat; suppurative lesions were common; younger rats on meat and vegetable diet did not develop the lesions
	Ham	1940 1941*	Mesentery and spleen	Lesions occurred in animals given a single massive dose of activated ergosterol which caused calcification in the kidneys, which in turn was thought by the author to have led to renal ischemia, hypertension and then periarteritis nodosa
	Wilson and Byrom	1941	Most marked in mesentery	Lesions occurred in 28 rats made hypertensive by placing a clamp on one renal artery; animals were well below the age limit of those used by Wilens and Sproul
	Friedman, Jarman and Klemperer	1941	Pancreas, mesentery, intestines, heart, testes and unwrapped kidney	Lesions described as periarteritis nodosa but not so designated; hypertension produced by cellophane-perinephritic method, using one kidney; inflammatory necrotizing arterial lesions in animals having rapid, severe rise in blood pressure
	Cromartie	1943	Widespread	Typical lesions were found in 15 of 37 rats in which hypertension had been produced by various operative methods; 12 of the 15 were infected; another 12 were infected but showed no periarteritis nodosa

* Personal communication.

tain kidney extracts. The dogs and rats were housed in laboratories in different buildings. The rats were fed Rockland Rat Diet, while the diet for the dogs consisted of dog biscuits, cooked meat scraps and bones, and occasionally milk.

Autopsies were performed on all of these animals and microscopic sections of heart, liver, spleen, kidney, pancreas, mesentery and occasionally other organs and tissues were studied.

Hypertension was induced in the rats by wrapping both kidneys with silk according to the technic described by Kempf and Page. Healthy rats of either sex, all under 6 months of age and weighing 150 to 200 gm., were used. The procedure of wrapping the kidneys was carried out in two stages: one kidney was wrapped with silk and 7 to 10 days later the other kidney was wrapped in similar fashion. Although the silk was autoclaved and the instruments soaked in alcohol, no special additional aseptic precautions were employed. Both stages of the operation were done under ether anesthesia, and the incisions closed with skin clips.

In rats, systolic arterial blood pressure determinations were carried out according to the indirect method of Williams, Harrison and Grollman. The blood pressure readings reported represent triplicate determinations after the animals had been heated in a box at 60° C. for 5 minutes. Blood pressure determinations were made usually three or more times a week.

Arterial blood pressure determinations in dogs were made by puncturing the femoral artery with an 18 gauge needle connected directly to a mercury manometer by pressure tubing. Such determinations were made weekly or oftener.

A number of the animals which became hypertensive were treated with kidney extracts designed to lower blood pressure. The extracts were prepared by modifications of the methods published by Page, Helmer, Kohlstaedt, Fouts, Kempf and Corcoran. These extracts were sterile by bacteriologic test and were administered orally, intramuscularly, or intraperitoneally, and in a few instances intravenously. The dosage varied according to the response elicited. An evaluation of the ability of these extracts to reduce the blood pressure will be presented separately. Evidence will be presented in this paper to show that treatment with these extracts did not cause periarteritis nodosa.

Results

The lesions were similar in rats and dogs and consisted of varying degrees of fibrinoid necrosis and inflammatory exudate in the walls of small and medium-sized arteries, particularly those of the mesentery, hilar regions of abdominal viscera, and in the epicardium of the right

ventricle. Acute, subacute and chronic stages were seen. The early phase consisted of fibrinoid necrosis of the media and adventitia with perivascular edema and the appearance of a few large cells resembling macrophages or histiocytes, which frequently contained many blue granules in the cytoplasm. The lesions spread to involve all three coats of the vessel walls (Fig. 1), producing a necrotizing panarteritis with pleomorphic inflammatory cellular exudation in which eosinophils were prominent. In more chronic cases, extensive granulomatous nodules were found in and around the vessel walls (Figs. 2 and 4). Frequently there was thrombosis of the lumina with varying degrees of vascular occlusion and aneurysm formation, which produced gross nodularity and angulation of vessels (Fig. 3). The mesenteric arteries were often enlarged to many times their normal caliber.

The process was largely confined to the small arteries; the arterioles only rarely were affected and when involved the lesions were not prominent. In a few cases with long-standing hypertension and extensive renal destruction, such as infarction, there was found the type of arteriolar necrosis described by Goldblatt and Kahn. Such lesions can be differentiated from those of periarteritis nodosa by the amount and type of inflammatory cellular exudation, the size of vessels involved and, to a lesser extent, by the distribution of the lesions. In arteriolar necrosis inflammatory reaction is rare, but when it occurs the cells are predominately neutrophils. In periarteritis nodosa inflammatory reaction is a prominent part even of early lesions, and the reaction is pleomorphic with neutrophils playing a minor rôle. In the abdominal viscera, arteriolar necrosis is most commonly seen in the nutrient arterioles within organs, while periarteritis nodosa is best seen in the smaller arteries in the hilum of an organ. In fact it is often limited to this site.

In the majority of the animals with periarteritis nodosa the lesions were widely distributed. Microscopic evidence of the disease was found in every organ except the lung. In a few cases of limited distribution the coronary and hepatic arteries were the vessels most frequently involved. For some unknown reason the arteries in the heart which most frequently presented lesions were those in the epicardium of the right ventricle about halfway between the apex and the atrioventricular junction. The size and histologic structure of vessels may be an important predisposing factor in the localization of the lesions.

Findings in Rats

In order to correlate various experimental factors with the occurrence of periarteritis nodosa, the 100 rats studied were divided into the following groups (the numbers of animals are noted):

A. Rats which were not treated with renal extracts and were not operated upon (28).

B. Rats which were treated with renal extract but were not operated upon (10).

C. Rats in which hypertension was induced by wrapping the kidneys with silk, but which received no renal extract (26).

D. Rats in which hypertension was induced by wrapping the kidneys with silk, but which subsequently received renal extract (36).

Group A served as controls for the other groups in regard to sex, age, strain, species, diet and environment.

Table II gives the number of cases of periarteritis nodosa found in

TABLE II

Occurrence of Periarteritis Nodosa in Animals Subjected to Various Controlled Procedures

	Number of animals	Periarteritis nodosa	
		Present,	Absent
Group A. No operation, no extract	28	0	28
Group B. No operation, extract treated	10	0	10
Total	38	0	38
Group C. Silk-perinephritis operation, no extract	26	6	20
Group D. Silk-perinephritis operation, extract treated	36	20	16
Total	62	26	36

each of the four groups of rats. Of the 38 which were not operated upon, none showed evidence of the disease either grossly or microscopically, while 26, or about 42 per cent of the animals which were operated upon, subsequently developed periarteritis nodosa. The odds against this association occurring by chance are greater than 16,000 to 1.*

Of the 62 rats with perinephritis induced by silk, 27 were males and 35 were females. The lesions of periarteritis nodosa were found in 14, or 52 per cent, of the males and in 12, or 34 per cent, of the females. This difference is too small to be considered significant with this number of animals.

In Table II the greater proportion of animals with periarteritis among those treated with extracts after operation is explained by the fact that extracts were given to the animals with the higher blood

* Computed according to the formula for a four-fold table as described by Raymond Pearl (Introduction to Medical Biometry and Statistics, W. B. Saunders Co., Philadelphia, 1930, ed. 2, p. 317).

pressures, and, as will be pointed out later in this report, animals with periarteritis tended to have higher blood pressures than the ones without it.

Since not all of the animals with silk-induced perinephritis developed periarteritis nodosa, the possible etiologic effect of the extracts was considered. Ten rats not operated upon received the extracts and none developed periarteritis nodosa. Thirty-six rats operated upon were subsequently given extracts. In 16, or about 45 per cent, periarteritis nodosa did not develop. Therefore, the extracts, either alone or in combination with the operation, failed to produce periarteritis nodosa in 26 animals. On the other hand, 6 rats which were operated upon but received no extracts developed periarteritis nodosa. It was concluded, therefore, that the treatment with extracts did not cause periarteritis in these rats. The possible effects of the extracts upon blood pressure will be discussed in a subsequent report.

Many of the rats of this series, both with and without periarteritis nodosa, presented extensive infectious processes, particularly suppurative lesions within the perinephric membrane. In those animals which presented periarteritis there was usually binding together of abdominal viscera by chronic granulomatous inflammation in which pockets of caseous necrosis and suppuration were found.

In the animals which did not present periarteritis nodosa the reaction around the silk was usually limited to a thin layer adjacent to the renal capsule, with very little or no involvement of the surrounding organs and tissues. Pockets of necrosis and infection were frequently not found. In some animals the reaction was so slight that the perinephric membrane was semitransparent. Sections of silk-wrapped kidneys were difficult to cut on the microtome; therefore it was considered impractical to make numerous microscopic sections of all kidneys to determine accurately the incidence of perinephric infectious processes.

Infections elsewhere in the body, such as lobular pneumonia, occurred occasionally in each group and were considered to be of no etiologic significance.

A distinct difference between the two groups of animals was disclosed by the blood pressure studies shown in Table III. In order to rule out the effect of possible errors in blood pressure readings, the mean pressures of the two groups were compared. In Table III the differences in the mean blood pressures between the rats with and without periarteritis are indicated. Statistical analysis permits the conclusion that the mean pressure of the periarteritic animals each month during

the period of observation was significantly higher than that of the non-periarteritic animals.*

The range of normal pressures was determined from 707 readings on 83 rats before operation or extract treatment. Ninety-nine per cent of the readings were between 100 and 139 mm. of mercury, the average being 122 mm.

TABLE III

Mean Blood Pressures of Rats Each Month after Last Operation

Interval after last operation		With periarteritis nodosa (A)	Without periarteritis nodosa (B)	Significance of differences $\left(\frac{\text{Mean}_A - \text{Mean}_B}{\sqrt{(\text{P. E.}_A)^2+(\text{P. E.}_B)^2}}\right)$
1st month	Mean pressure and P. E. Number of pressures Number of animals	178.7 ± 0.8 224 22	167.8 ± 0.4 345 33	12.2
2nd month	Mean pressure and P. E. Number of pressures Number of animals	176.6 ± 0.6 409 24	165.0 ± 0.4 422 32	16.1
3rd month	Mean pressure and P. E. Number of pressures Number of animals	175.0 ± 0.6 326 22	165.3 ± 0.5 195 26	12.4
4th month	Mean pressure and P. E. Number of pressures Number of animals	173.4 ± 0.7 164 15	166.9 ± 0.7 143 16	6.6
5th month	Mean pressure and P. E. Number of pressures Number of animals	176.1 ± 1.1 100 6	168.9 ± 0.9 95 10	5.1
6th month	Mean pressure and P. E. Number of pressures Number of animals	189.1 ± 1.3 78 5	168.0 ± 0.8 69 6	13.8

Findings in Dogs

Eight dogs were operated upon. One kidney was wrapped with silk and the other kidney removed. Of these animals four developed periarteritis and only one of these received renal extract. The other four did not develop periarteritis nodosa.

As shown in Table IV, the blood pressures of dogs with periarteritis were decidedly higher than of those without this condition.

In three of the dogs with periarteritis there were suppurative foci in the perinephric membrane and beginning abscess formation in the renal substance.

In the fourth animal in which periarteritis nodosa was limited to the coronary arteries, no evidence of infection was found in the sections studied. This animal died suddenly 3 weeks after operation, having

* A difference which is three or more times its probable error is considered significant (Pearl, *loc. cit.*).

developed hypertension (215 mm. Hg) 1 week after operation. The cause of death was extensive myocardial necrosis and hemorrhage.

The four dogs which did not develop periarteritis had mild hypertension with no evidence of suppuration in the kidney or perinephric membrane. Two of the four had slight arteriolosclerosis in some of the viscera.

TABLE IV

Monthly Average Blood Pressures of Dogs
(Mm. of Mercury)

Interval after last operation	Mean pressure, probable error and number of readings							
	Dogs without periarteritis nodosa				Dogs with periarteritis nodosa			
	No. 255	No. 360	No. 392	No. 398	No. 314	No. 348	No. 358	No. 367
1st month	155 ± 3 (8)	160 (2)	160 (1)	145 ± 3 (6)	151 ± 8 (10)	210 ± 3 (3)	207 ± 13 (3)	210 (2)
2nd month	145 ± 3 (7)	168 (2)	170 (2)	145 (2)	199 ± 4 (6)		248 ± 4 (6)	275 ± 2 (4)
3rd month	180 ± 8 (3)	140 (2)		165 (1)			238 ± 3 (17)	
4th month	185 (1)		205 (1)				228 ± 5 (12)	
5th month			285* (1)					
6th month	160 (1)							
7th month	155 (1)	125 (1)						
8th month	140 (1)	135 ± 5 (4)						
9th month	173 ± 6 (3)	150 ± 1 (11)						
10th month	163 ± 2 (11)	148 ± 3 (6)						
11th month	172 ± 2 (10)	150 (1)						

* Died on the following day.

Periarteritis nodosa has never been encountered in the large group of dogs which have lived in the same laboratory as the above animals and which have been studied for other purposes.

COMMENT

In reviewing previous reports concerning the occurrence of periarteritis nodosa in animals it has been difficult to attribute the condition to any one factor.

The occurrence of this condition in epidemic form in the herd of axis deer cited in Table I suggests an infectious agent. However, the majority of investigators seem to think that periarteritis nodosa is not a specific disease but represents a reaction (possibly allergic) of the arterial system to a variety of infections and toxemias. Rich and Gregory have reported the occurrence of this vascular lesion in human

patients who have manifested hypersensitive reactions to serums or to a sulfonamide. They also described typical lesions in rabbits in which hypersensitivity had been induced by the administration of horse serum. Most of their animals also had acute diffuse glomerulonephritis. They described no controls or blood pressure studies.

Wilson and Byrom have noted periarteritis nodosa in certain rats made hypertensive by clamping the renal artery, and believe that the factor determining the lesion is the sudden strain imposed on the vessel wall by marked vasoconstriction and the resultant sudden rise in blood pressure. These authors did not investigate the possible relation between periarteritis and the presence of infection in their animals. Cromartie's rats, which developed periarteritis, were all hypertensive and most of them presented infection around the kidneys. Since some human patients with periarteritis nodosa apparently have not had hypertension, it seems that other factors may play a rôle in the genesis of this condition in man.

Since blood pressure levels of the two groups of hypertensive rats of the present series were essentially the same before operation and were within normal limits, it would be interesting to know how soon a significant difference began to appear. Table III shows that the rise in blood pressure following the production of perinephritis by silk had reached a fairly constant level by the end of the first month after operation. Also, this blood pressure level was decidedly higher in rats which developed periarteritis nodosa.

The question arises as to which developed first, the higher blood pressure or the periarteritis. Data are not available to answer this question since the blood pressure of these animals was not measured for 2 weeks following the second stage of the operation. Also, the possible rôle played by hypersensitivity has not been determined. These problems are now being investigated in additional series of animals.

Summary

1. Periarteritis nodosa was found at autopsy in 26 of 62 rats and 4 of 8 dogs which had been made hypertensive by wrapping their kidneys with silk. No evidence of these lesions was found in groups of control animals.

2. In animals presenting periarteritis nodosa at autopsy, the monthly mean blood pressure levels had been higher than in animals in which no periarteritis nodosa was found. This higher level had been manifested within 1 month after the production of perinephritis and had been maintained throughout the 6 months of observation.

3. Suppurative lesions were common in the experimentally-produced perinephric membranes.

4. A review of the literature revealed no report of the occurrence of periarteritis nodosa in animals in which the kidneys and the blood pressure were proved to be normal.

5. In the present series of hypertensive animals the two observed differences between those which had and those which did not have periarteritis nodosa were, in the former: (1) higher mean blood pressure levels, and (2) more frequent and more extensive suppurative lesions around the kidneys.

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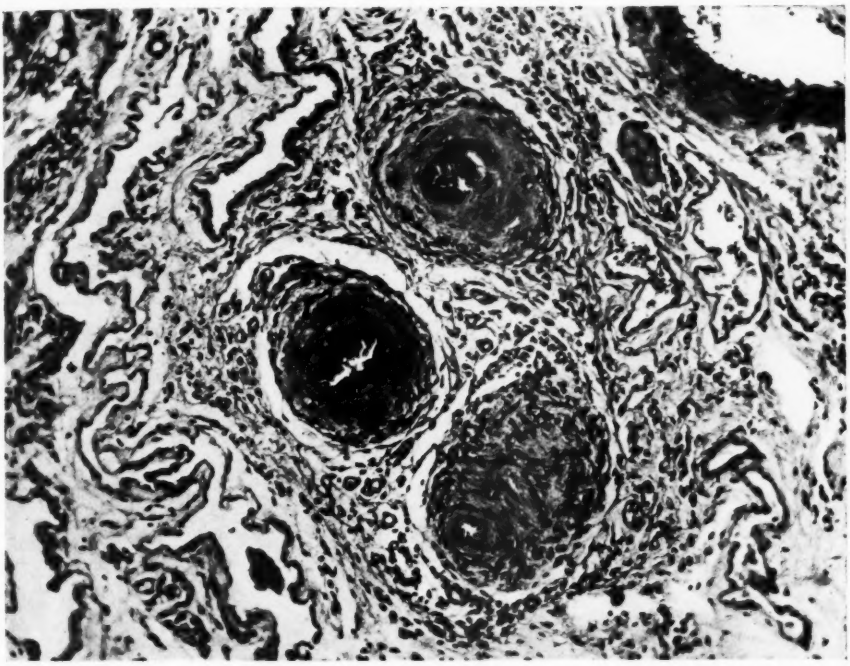
DESCRIPTION OF PLATES

PLATE 139

FIG. 1. Renal pelvis of a rat showing necrotizing periarteritis nodosa with beginning granulomatous changes. × 149.

FIG. 2. Mesentery of a rat showing marked granulomatous changes of periarteritis nodosa. × 78.

1



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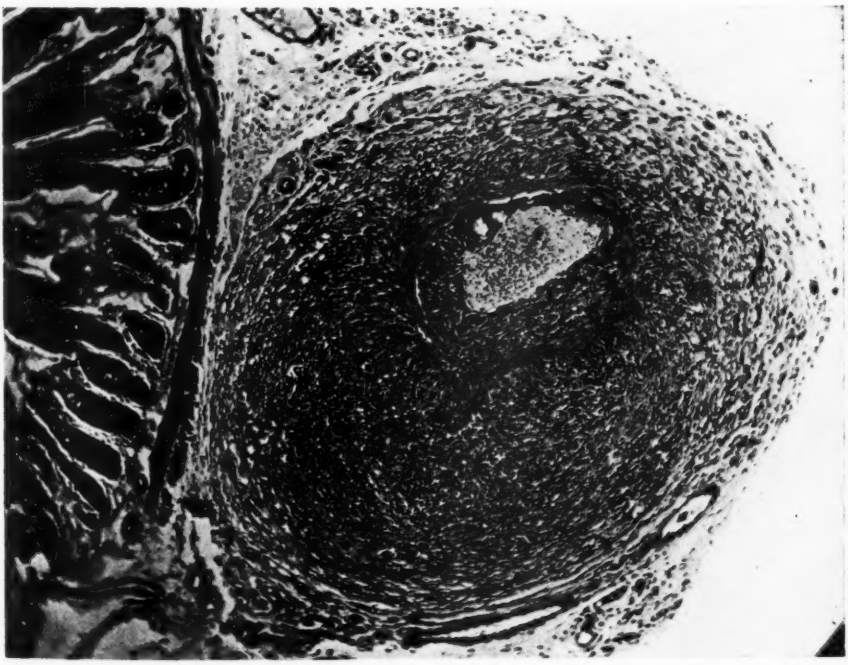

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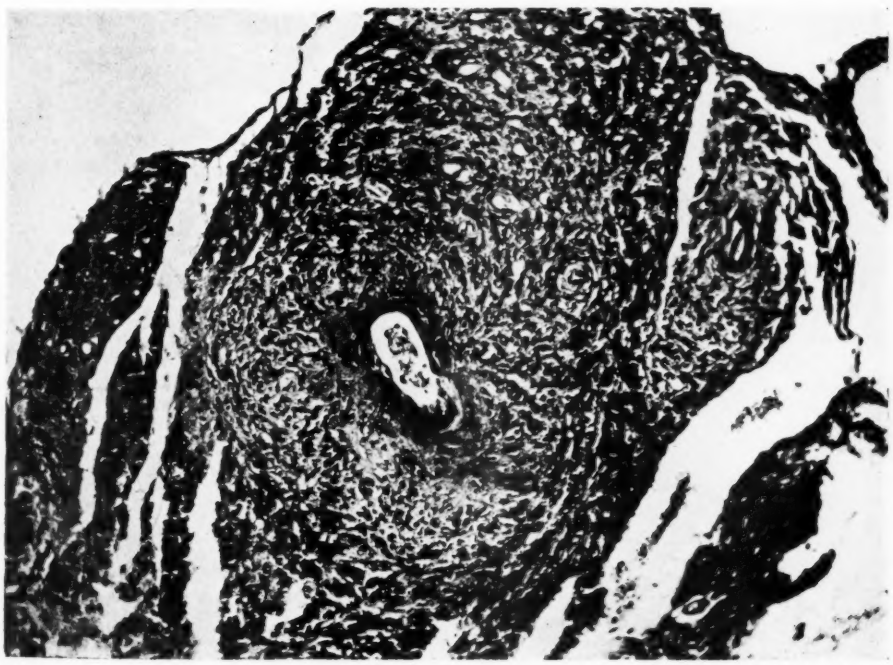
PLATE 140

FIG. 3. Loop of the small intestine of a rat showing typical lesions in the mesenteric arteries.

FIG. 4. Heart of a rat showing periarteritis nodosa in wall of right ventricle. × 140.

3

4



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REACTIONS OF THE BLOOD AND ORGANS OF DOGS AFTER INTRAVENOUS INJECTIONS OF SOLUTIONS OF METHYL CELLULOSES OF GRADED MOLECULAR WEIGHTS *

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The intravenous introduction of macromolecular substances of varying molecular weights, sizes and shapes (plasma, plasma albumin, plasma globulin, hemoglobin, gelatin, isinglass, polyvinyl alcohol, methyl cellulose, gum arabic, pectin,[1-9] vinyl pyrrolidon polymer[10]), or of colloidal material of different particle sizes (Evans blue, Congo red, inulin, metallic colloids) is a procedure used for therapeutic purposes in shock, hemorrhage and septic infections as well as for diagnostic purposes in the determination of total plasma volume, amyloidosis and renal function. Disturbances of the macromolecular and colloidal status of the proteins, polysaccharides and lipids of the blood are found in immune reactions, myelomatosis, glycogen-storage disease, nephrosis, and diseases with lipemic reactions.

Despite the obvious frequency of abnormal fluctuations in the macromolecular, colloidal equilibrium of the blood, no systematic investigations exist concerning the rôle which the size of the aggregates eliciting such reactions plays in the development and the type of the pathological manifestations produced in the blood and organs. The present investigations were conducted in an attempt to fill this gap by using a series of methyl celluloses which are identical in chemical structure, but differ in the relative length of the dextrose chain and thus in molecular size.

Experimental Procedure

Inasmuch as detailed information on the physicochemical properties of methyl cellulose has been recorded in previous publications (Hueper,[4] Hueper and Ichniowski,[9] Hueper, Martin and Thompson[11]), it may suffice here to point out that methyl cellulose is the methyl ether of cellulose. As a hydrophilic colloid, it forms a viscous solution, from which it can be precipitated by the addition of various salts and which forms a firm, white coagulum upon heating above 65° C., reversible on cooling. It is digested by pancreatin at 40° C. with the development of reducing sugars.[12] When introduced into the blood, it forms an emulsion with the blood plasma. Table I presents the relations which exist as to grade of viscosity, molecular weight and degree of polymeri-

* Received for publication, August 30, 1943.

zation between the seven types of methyl cellulose used in this and preceding studies.

The data on the molecular weights, the degree of polymerization and the grade of viscosity for the 2 per cent solution were supplied by Dr. R. M. Upright, The Dow Chemical Company, from which the various methyl celluloses were obtained. The viscosity of the 0.25 per cent solution was determined with the Hess' viscosimeter and compared with water at 18.5° C.

The present studies were concerned mainly with six members of the series of methyl celluloses, since methyl cellulose of 400 cps. (centipoises) was employed in previous investigations (injected as a 2 per cent solution in amounts of from 25 to 130 cc. into 7 dogs). The data

TABLE I

Physicochemical Properties of Methyl Celluloses

Viscosity grade		Average molecular weight	Polymerization degree
2% sol. cps.*	0.25% sol. H_2O		
15	1.45	32,200	169
25	1.55	36,400	191
50	1.85	53,400	281
100	2.05	60,500	318
400	2.73	77,700	409
1500	3.95	118,200	622
4000	6.00	143,600	756

* Centipoises.

obtained on those occasions, therefore, will be mentioned only briefly for comparative purposes. The solutions of the various methyl celluloses were prepared in 1 per cent sodium chloride solution and had a viscosity of approximately 16 or 8 times that of normal plasma (methyl cellulose, 15 cps., 2.8 per cent; m.c., 25 cps., 2.4 per cent; m.c., 50 cps., 2 per cent; m.c., 100 cps., 1.8 per cent; m.c., 1500 cps., 1 per cent; m.c., 4000 cps., 0.7 per cent). While methyl celluloses of 1500 cps. and 4000 cps. gave a water-clear solution when dissolved at icebox temperature, the solutions of the other methyl celluloses were more or less turbid and had to be filtered through filter paper and Hyflo Supercel* for the removal of coarser floccules. They remained slightly turbid even after these procedures.

For the study of the more immediate hematic reactions, 40 cc. of these solutions were injected intravenously into dogs. There were 2 or 3 dogs in each group. The observation time of these reactions was 24 hours. The prolonged experiment started immediately thereafter

* Hyflo Supercel is a preparation of purified diatomaceous earth, obtained from the Dicalite Co., 120 Wall Street, New York, N.Y.

and consisted in the intravenous injection of methyl cellulose solution 5 days per week in rising amounts. The daily dose was 40 cc. during the first week, and was increased by 10 cc. each week until 130 cc. was reached for those dogs which survived longest. The average weight of the 18 dogs used in these and previous experiments was 9.3 kg. and the weight range was between 7 and 12 kg. The observation time varied for the different dogs from 1 week to 6 months. Examinations of the blood (hemoglobin, number of erythrocytes and leukocytes, coagulation time, sedimentation rate, volume of packed cells, plasma viscosity) were made at intervals of 2 weeks. Autopsies were performed on all dogs.

A. Hematic Reactions after the Intravenous Injection of a Single Dose of Methyl Cellulose Solution

Examinations of the blood were made 5, 15, 30, 60, 120, 240 minutes and 24 hours after the injection, following the standard determinations.

a. Methyl Cellulose, 15 cps. (Two Dogs)

Erythrocytes. During the first 120 minutes there occurred in both dogs moderate to severe downward fluctuations of the number of erythrocytes, amounting to from 1.5 to 3 million cells. Thereafter the number of erythrocytes became stationary at a level slightly to moderately below the original one.

Hemoglobin and Volume of Packed Cells. Variations in hemoglobin and volume of packed cells ran parallel to those of the erythrocytes.

Leukocytes. There was a moderate to severe leukopenia 5 to 15 minutes after the injection, followed by return to approximately the original level within 2 hours.

Clotting Time. There were no significant deviations in clotting time.

Sedimentation Rate. There occurred a marked acceleration of the erythrocytic sedimentation rate before 4 hours after the injection. This was followed by a considerable decrease at the 24 hour test.

Plasma Viscosity. The plasma viscosity was moderately increased (0.5 to 0.1) beyond the normal range immediately after the injection, and returned to normal at the 4 hour test.

b. Methyl Cellulose, 25 cps. (Two Dogs)

The hematic reactions followed closely in pattern those seen in the animals of the previous group, with the exception of the sedimentation rate which was already markedly increased at the 5 minute test, and remained high to the end of the observation period (24 hours).

c. Methyl Cellulose, 50 cps. (One Dog)

Erythrocytes. Following an increase by 1.35 million at the 5 minute level, the erythrocytes remained relatively stationary around the original level during the 24 hour observation period.

Hemoglobin and Packed Cell Volume. The hemoglobin values, as well as the figures obtained from hematocrit determinations, remained below the original level throughout the 24 hours, exhibiting a tendency to drop progressively.

Leukocytes. Transitory leukopenia reached its lowest level at the 15 minute test. The leukopenic crisis had completely disappeared at the 4 hour test.

Clotting Time. No significant changes in clotting time were found.

Sedimentation Rate. Sedimentation was definitely hastened at the 5 minute test, and was moderately accentuated during the 24 hour period.

Plasma Viscosity. There was a marked increase in plasma viscosity directly after injection (1.25). This reaction subsided somewhat during the first 4 hours, at the end of which the increase in viscosity had dropped to 0.35; but this was followed by a renewed increase after 24 hours by 1.6 points (to 3.75) above the original level (2.15).

d. Methyl Cellulose, 100 cps. (Three Dogs)

Erythrocytes. With the exception of 1 dog which showed an initial rise in the number of the erythrocytes at the 5 minute test, amounting to 1.15 million, the erythrocytes remained relatively stationary during the first 2 hours and decreased moderately in 2 dogs from then on.

Hemoglobin and Volume of Packed Cells. There was a definite tendency to reduced values for both hemoglobin and volume of packed cells with the progress of the test.

Clotting Time. No deviations from the normal range of clotting time were noted.

Sedimentation Rate. Sedimentation of erythrocytes was definitely accelerated at the 5 minute test, and was accentuated during the further course of the experiment. The sedimentation rate thus remained persistently high during the 24 hour observation period.

Plasma Viscosity. The plasma viscosity was moderately increased immediately after the injection (0.65 to 1.0 point) and remained elevated during the entire period.

e. Methyl Cellulose, 400 cps. (Three Dogs)

Erythrocytes. There was a mild reduction in erythrocytes during the first 4 hours, followed by a return to the original level at the 24 hour test.

Hemoglobin and Volume of Packed Cells. The hemoglobin values dropped from the 5 minute test on, and remained reduced during the entire 24 hour period. Hematocrit values followed a similar trend.

Leukocytes. Transitory leukopenia lasted from 2 to 4 hours, and was followed by leukocytosis at the 24 hour test.

Clotting Time. There was a lengthening of the clotting time appearing 15 minutes to 2 hours after the injection and persisting in 2 dogs at the 24 hour test.

Sedimentation Rate. Acceleration of erythrocytic sedimentation was marked at the 5 minute test and persisted for more than 24 hours.

Plasma Viscosity. Plasma viscosity was markedly increased immediately after injection and then showed a moderate drop, but remained considerably above the original level during the entire 24 hours.

f. Methyl Cellulose, 1500 cps. (Three Dogs)

Erythrocytes. There was no consistent or appreciable variation in the number of red cells during the first 15 minutes. All dogs showed during some time thereafter a moderate reduction in erythrocytes.

Hemoglobin and Volume of Packed Cells. Fluctuations in hemoglobin and volume of packed cells paralleled in general those in the number of red cells.

Leukocytes. Transitory leukopenia lasted for 4 hours or more.

Clotting Time. There was a minor to moderate increase in the clotting time during the first hour with a return to normal values thereafter.

Sedimentation Rate. The hastening of the erythrocytic sedimentation was pronounced at the 5 minute test and remained high or even increased during the remainder of the observation period.

Plasma Viscosity. Increase in the plasmatic viscosity was mild and did not persist for longer than 30 minutes.

g. Methyl Cellulose, 4000 cps. (Three Dogs)

Erythrocytes. Following a moderate brief reduction in erythrocytes some time during the first 45 minutes, the number of red cells returned to approximately the original level for the remainder of the 24 hour period.

Hemoglobin and Volume of Packed Cells. The hemoglobin was comparatively decreased, particularly during the latter part of the experimental period. The hematocrit values showed a similar movement.

Leukocytes. Transitory leukopenia extended beyond the 4 hour test. A moderate to marked leukocytosis was found in 2 dogs at the 24 hour examination.

Clotting Time. No significant deviations in clotting time were observed.

Sedimentation Rate. The increase in erythrocytic sedimentation was moderate during the first 45 minutes and became considerable and persistent thereafter.

Plasma Viscosity. There was a mild increase in the plasmatic viscosity not surpassing the normal range, which persisted throughout the experimental period.

The hematic reactions observed in dogs following the intravenous introduction of 40 cc. of solutions of the seven types of methyl cellulose prepared in normal saline solution and having the same viscosity (8 times that of plasma) revealed the following pattern and discrepancies. The number of erythrocytes exhibited in all instances a tendency toward a mild to moderate decrease, particularly toward the latter part of the observation period. This movement was displayed more consistently and considerably by the amount of hemoglobin and the volume of packed cells, especially with the methyl celluloses of higher molecular weight. A leukopenic reaction was noted with all types of methyl cellulose, but the leukopenic crisis was least persistent with the methyl celluloses of low molecular weight, while lasting for more than 4 hours with those of higher molecular weights, in connection with which the reaction was not infrequently followed by a leukocytosis at the 24 hour test.

Abnormalities in clotting time were found only with methyl celluloses of 400 and 1500 cps. and especially with the former. The sedimentation rate was increased with methyl celluloses of all types. However, this acceleration showed a delayed appearance with the two low molecular methyl celluloses, compared with the immediate hastening found with the other types. It was noted, moreover, that this response was of lesser duration with the low molecular products, whereas it was of higher severity and greater duration with the higher molecular substances. The plasma viscosity was increased in all instances immediately after the injection. This increase was of relatively short duration and low intensity with the two low molecular methyl celluloses. It was of higher intensity and long duration with the medium molecular substances and of low intensity and long duration with those of high molecular weights, while no pathological increase resulted from the injection of the methyl cellulose of highest molecular weight.

B. Hematic Reactions after the Intravenous Injection of Repeated Doses of Methyl Cellulose Solution

Blood tests were made at intervals of 2 weeks, unless severe changes necessitated a more frequent control of the status of the blood.

a. Methyl Cellulose, 15 cps. (Two Dogs)

One dog received within 2 weeks a total of 570 cc. of methyl cellulose solution, while the second dog was injected within 18 days with 670 cc. Both dogs died.

Erythrocytes. There was a moderate decrease in the number of red cells, ranging from 1.1 to 1.8 millions below the original value.

Hemoglobin and Volume of Packed Cells. The decrease in hemoglobin and in volume of packed cells comparatively surpassed that of the erythrocytes to a minor degree.

Leukocytes. There were no pathological fluctuations in the number of leukocytes.

Clotting Time. No abnormal deviations in clotting were noted.

Sedimentation Rate. There was a marked increase in the sedimentation rate.

Plasma Viscosity. Toward the end of the experimental period the plasma viscosity was elevated by 50 to 100 per cent above its normal level.

b. Methyl Cellulose, 25 cps. (Two Dogs)

One dog received a total of 1580 cc. of methyl cellulose solution within 52 days, whereas the second dog was injected with 130 cc. within 11 days. Both dogs died.

Erythrocytes. The red cells dropped by more than 66 per cent in the dog surviving for 52 days (from 6 million to 1.65 million).

Hemoglobin and Volume of Packed Cells. The reduction in the amount of hemoglobin was approximately equal to that of the erythrocytes, while the decrease in the volume of the packed cells even surpassed the drop of the erythrocytes.

Leukocytes. There was a temporary moderate leukocytosis followed during the latter part of the experiment by a return to normal values.

Clotting Time. No abnormalities in clotting time were noted.

Sedimentation Rate. The sedimentation of the erythrocytes was highly and increasingly accelerated.

Plasma Viscosity. The plasma viscosity rose to a peak of 4.9, 4 weeks after the start and then gradually dropped back to 3.25, rising again toward the end to 4.5.

c. Methyl Cellulose, 50 cps. (Four Dogs)

One dog was injected with 290 cc. of methyl cellulose solution within 10 days, a second with 370 cc. within 12 days, a third with 1320 cc. within 32 days, and a fourth with 2010 cc. within 75 days. All dogs died.

Erythrocytes. In all dogs there was a progressive, and ultimately considerable, decrease in the number of erythrocytes. In the dog sur-

viving 75 days the drop was from 6.21 million to 1.42 million, following a temporary partial recovery during a period in which the injections were discontinued.

Hemoglobin and Volume of Packed Cells. The reduction in hemoglobin was considerable and progressive and reached in the aforementioned dog a level of less than 5 gm. per 100 cc. of blood. The decrease in the volume of packed cells was relatively more pronounced than the decrease of red cells in those animals having a moderate anemia, while it was comparatively less definite in the dog with the severe anemia after having passed previously through the first mentioned relation.

Leukocytes. There was a moderate leukocytosis present during the latter part of the experimental period.

Clotting Time. There were no abnormalities in clotting time.

Sedimentation Rate. There was a very marked acceleration of the sedimentation rate (75 to 79 mm., Wintrobe tube).

Plasma Viscosity. The plasma viscosity was considerably elevated, varying in 3 dogs between 4.9 and 6.0, after having undergone during the early part of the experiment a considerable increase followed during the middle part by a decrease to slightly elevated levels when the injections were discontinued and subsequently again by a marked elevation following the resumption of the treatment.

d. Methyl Cellulose, 100 cps. (Three Dogs)

One dog received 80 cc. within 7 days, a second dog was injected with 4645 cc. within 90 days, and a third dog with 5190 cc. within 97 days. All died.

Erythrocytes. After 2 weeks of treatment there developed a rapidly progressive and severe reduction in the number of red cells, causing after 2 months a drop to one-third and one-fourth, in the respective surviving animals, of the original number. When the treatment was then discontinued, there followed a rapid rise in the number of erythrocytes, which amounted to from 2 to 2.5 million within 2 to 3 weeks. There was, however, a very rapid drop in erythrocytes when the injections were again given, reaching values of 2.4 and 1.35 million, respectively, before death.

Hemoglobin and Volume of Packed Cells. The hemoglobin followed in general the fluctuations of the erythrocytes and sank ultimately below 5 gm. per 100 cc. of blood. The volume of packed cells dropped during the first half of the erythrocytic decrease, comparatively very sharply surpassing the reduction of the red cells, while during the latter part of the experiment the decrease in the volume of packed cells was relatively smaller than that of the erythrocytes.

Leukocytes. There were no significant deviations in the number of leukocytes from the normal range, except during the final days, when a moderate leukocytosis existed.

Clotting Time. No abnormalities in clotting time were noted.

Sedimentation Rate. An early and marked acceleration of erythrocytic sedimentation persisted through the treatment-free period to the end.

Plasma Viscosity. The plasmatic viscosity was increased to slightly above 6 during the major part of the experiment, except during the treatment-free period, when the viscosity dropped to values between 2.5 and 3.5.

e. Methyl Cellulose, 400 cps. (Seven Dogs)

One dog received 260 cc. of methyl cellulose solution within 6 days, a second and a third animal were injected with 1040 cc. within 12 and 13 days respectively, a fourth dog was injected with 2112 cc. within 57 days, a fifth received 2930 cc. within 66 days, a sixth 3375 cc. within 83 days, and a seventh 5720 cc. within 6 months. The first 3 dogs died, while the other 4 were killed. The seventh animal was sacrificed 2 months after the last injection, while the others were sacrificed not later than a few days after the last treatment.

Erythrocytes. There occurred a gradual decrease in the number of erythrocytes, which reached, however, severe degrees only in those animals which died, remaining within a moderate range in those which survived the prolonged treatment for many weeks and were finally sacrificed. It was noted, moreover, that during a temporary discontinuation of the treatment a relatively rapid increase in the number of red cells developed. This was especially striking in the dog which was killed 2 months after the last injection and which showed a recovery to the original erythrocytic level 1 week after cessation of treatment.

Hemoglobin and Volume of Packed Cells. The hemoglobin decreased during the early part somewhat more rapidly than the erythrocytes, but exhibited a normal relation to the cellular elements in the latter part of the experiments. The volume of packed cells underwent a similar movement.

Leukocytes. The number of white cells fluctuated within the normal range, sometimes reaching relatively low figures.

Clotting Time. The clotting time was rather consistently and considerably increased, and decreased slowly after cessation of treatment.

Sedimentation Rate. Erythrocytic sedimentation was considerably hastened during the entire time of treatment and showed a gradual return toward normal values after cessation of injections.

Plasma Viscosity. The plasma viscosity was markedly increased during the course of treatment and particularly so during the last days of those dogs which died spontaneously. In those animals it reached values up to 7.88 after relatively few injections. In dogs with more prolonged and severe treatment this value was often surpassed during the more advanced stages, reaching 9.68. The viscosity remained at a high level for weeks after the cessation of injections, showing a slow decline.

f. Methyl Cellulose, 1500 cps. (Three Dogs)

One dog received a total of 990 cc. of methyl cellulose solution within 4 weeks. A second dog was injected with 2330 cc. within 7 weeks and a third with 4590 cc. within 11 weeks. All died.

Erythrocytes. The number of erythrocytes decreased after prolonged treatment to less than one-third of the original value.

Hemoglobin and Volume of Packed Cells. The hemoglobin was considerably reduced and reached in 2 dogs values below 5 gm. per 100 cc. of blood. The decrease of hemoglobin thus surpassed that of the erythrocytes. The reduction in the volume of packed cells, on the other hand, was somewhat less in degree than that of the erythrocytes.

Leukocytes. The leukocytes were always either at a high normal level or slightly above that range.

Clotting Time. No abnormalities in clotting time were found.

Sedimentation Rate. There was a marked hastening of the erythrocytic sedimentation.

Plasma Viscosity. The plasma viscosity was only moderately increased, reaching as peak value a viscosity of 4.

g. Methyl Cellulose, 4000 cps. (Three Dogs)

One dog received 2290 cc. of methyl cellulose within 6 weeks, a second was injected with a total of 3440 cc. within 8 weeks, and a third dog with 4930 cc. within 12 weeks. All died.

The hematic changes were similar to those seen in the series injected with methyl cellulose, 1500 cps. This was particularly so in regard to the increase in plasma viscosity, which remained below 3.5. It is remarkable that the dog which received 2290 cc. of methyl cellulose developed an anemia of 850,000 erythrocytes and a leukocytosis of 100,000 cells 3 days before death. In the dog which was injected with 4930 cc. the final erythrocytic number was 1.45 million (originally 8 millions) and the final leukocytic number, 42,600 cells.

The hematic reactions noted after repeated intravenous injections of the seven types of methyl cellulose exhibited a uniform pattern in many respects, but certain important deviations in others. There was in all

instances a considerable decrease in the number of the circulating erythrocytes, in the amount of hemoglobin and in the volume of packed cells, which was especially pronounced with the higher molecular types. The reduction of the last two items usually surpassed in degree that of the erythrocytes. It was evident, moreover, from the instances in which the treatment was interrupted for some time, that this anemia-producing effect was relatively readily reversible if the further introduction of the causative agent was stopped. As a rule, the leukocytes stayed within the normal range, but sometimes were slightly to moderately increased during the final stage. This reaction was especially pronounced in the dogs injected with m.c., 4000 cps., one of which exhibited a marked hyperleukocytosis of leukemoid type. The clotting time underwent abnormal changes only in animals injected with m.c., 400 cps., in which it was considerably lengthened. The sedimentation rate was definitely accelerated in all instances and remained so for some time after discontinuation of treatment. The plasma viscosity was always increased. However, this increase was comparatively small with the methyl celluloses of the low and the high molecular weights (m.c., 15 cps., 25 cps., 1500 cps., 4000 cps.), while it was highest with methyl cellulose of 400 cps. having a molecular weight approximating that of serum albumin. It was noted, moreover, that the elevated viscosity dropped rather rapidly to slightly increased values after discontinuation of treatment in dogs injected with methyl celluloses of 50 cps. and 100 cps., whereas it stayed considerably elevated for periods of many weeks after cessation of injections in dogs treated with methyl cellulose of 400 cps.

C. Anatomical Reactions in the Internal Organs Following Repeated Injections of Methyl Cellulose

a. Methyl Cellulose, 15 cps. (Two Dogs)

The 2 dogs of this series received 570 and 670 cc. of methyl cellulose solution respectively, and showed at autopsy practically normal organs with the exception of a large pneumonic induration in the left upper lobe of 1 dog. The spleens were of normal size, of firm consistency and grayish pink.

The histological study of the various organs showed the following findings:

Thyroid; Aorta; Pulmonary Artery; Large Elastic Arteries; Muscular Arteries of Thyroid, Kidney and Adrenal; Pancreas; Stomach and Intestine; Bladder. Normal.

Lung. Purulent bronchitis and large bronchopneumonic foci were present in the lungs of both dogs. There were deposits of a homogene-

ous, vacuolated, light-blue-stained matter in the peribronchial tissue spaces of 1 dog.

Heart. Small bluish-colored homogeneous deposits were found in the myocardial interstices.

Liver. Pericentral fatty degeneration or necrosis of liver cells.

Adrenals. Small bluish deposits were present in the sinusoids of the adrenals of 1 dog.

Spleen. The pulp consisted of accumulations of small foam cells, many of them in a stage of disintegration. Follicles were atrophic.

Kidney. The cortical tubules were distended, cystic, and lined by flattened epithelium. Some of the glomeruli showed grape-like, distended, cystic, capillary loops containing foam cells, or, occasionally were lined by a multinucleated syncytium, which was found also in the lining of some tubules. The interstitial tissue of 1 dog contained bluish homogeneous deposits in the interstices. Renal vessels were normal.

Testes. Arrest of spermatogenesis was present in both dogs. This was associated in 1 dog with the presence of some spermatid giant cells and interstitial edema and was accompanied in the second dog by a severe atrophy of the spermatogenic epithelium, leaving only the spermatogonia and the Sertoli's cells preserved. Some of the tubules were collapsed.

Bone Marrow. There was in the sternum a loose, hyperemic, immature myeloid tissue.

b. Methyl Cellulose, 25 cps. (Two Dogs)

The two dogs of this series received a total of 350 and 1580 cc. of methyl cellulose solution respectively, and showed at autopsy, apart from emaciation, normal internal organs.

The histological examination of the organs revealed the following findings:

Brain; Hypophysis; Parathyroid. Normal.

Thyroid. The follicles contained a thin colloid or were empty. In 1 dog the epithelial lining was desquamated in some follicles. The thyroid of the second dog which received the larger dose of methyl cellulose was normal.

Lung. The lungs were highly edematous, congested and hemorrhagic in the first dog and normal in the second dog.

Heart. The heart was normal in the first dog. The walls of the subepicardial coronary arteries in the heart of the second dog were transformed, particularly in their intimal portion, into a thick foam-cellular tissue considerably narrowing the lumina. Similar lesions were found in the myocardial arteries and arterioles. The subendocardial myo-

cardium of the left ventricle, especially of the papillary muscles, contained large and numerous calcium incrustations of necrotic muscle cells in addition to foci of loose fibrous tissue replacing muscle tissue (Fig. 1).

Aorta. The ascending aorta of the first dog showed a granular necrosis in the thickened intima and adjacent media. In a nearby segment the outer and middle media contained large hyaline areas with an extensive nodular calcification causing a bulging of the vessel wall into the lumen. The subintimal media was edematous. In the thoracic aorta there was perivascular hyalinization around vasa vasorum in the middle media. The aorta of the second dog exhibited similar and extensive medial hyalinization and calcification in the media of the aortic bulb, which showed, moreover, a thin foam-cellular coating and small foam-cellular cushions of the intima. In the thoracic part of the aorta extensive lesions of these types were present. The vasa vasorum of the adventitia were partly obliterated by foam-cellular intimal proliferations, while large portions of the media were hyalinized.

Pulmonary Artery. There were areas of medial calcification above the valves together with some foam-cellular intimal cushions in the second dog.

Large Elastic Arteries. Some large elastic arteries of the second dog showed extensive calcification of the media in some areas and in other parts incrustation of the elastic fibrils together with cyst formation. In several segments these lesions were associated with foam-cellular proliferation of the intima (Fig. 2).

Stomach. There was a diffuse fine granular calcification in the interstitial tissue of the mucosa in the second dog.

Liver. Extreme congestion and extensive fresh coagulation necrosis of the liver cells characterized the lesions of the first dog. The Kupffer cells of the second dog were markedly increased in number and had foamy cytoplasm. The cell cords were pushed apart thereby and the hepatic cells were moderately atrophic.

Pancreas. A medium-sized artery in the normal pancreatic parenchyma of the second dog showed intimal foam-cellular cushions and medial subendothelial calcium deposits.

Spleen. The organ was normal in the first dog and showed a pulp composed of foam cells in the second dog.

Adrenals. These organs were normal in the first dog and contained foam-cellular reticulo-endothelial cells of the medulla in the second dog.

Kidney. The tubules of the cortex were distended, cystic, and lined with flattened cells in both dogs. The glomeruli were hypertrophic and showed extensive hyalinization in the first dog. In the second dog many

consisted of a few cystic structures lined by foam cells or by a multinucleated syncytium. The arterioles in the kidney of the first dog had a distinct, thick, red-stained subintimal layer, while the arteries of the second dog showed a few foam-cellular cushions of the intima undergoing degenerative changes.

Testes. The spermatogenic epithelium of the tubules was reduced in the second dog to the spermatogonia and Sertoli's cells with a few large giant cells. The arterioles revealed a hyaline subendothelial thickening.

Epididymis. The ducts were empty.

Bone Marrow. The sternum contained a dense, immature myeloid tissue.

c. Methyl Cellulose, 50 cps. (Three Dogs)

One dog was injected with 370 cc. of the methyl cellulose solution, a second with 1120 cc. and a third with 2010 cc. The lungs were edematous, hyperemic and studded with dark red, hemorrhagic foci. The spleens of the second and third dogs were enlarged, soft and grayish pink. The livers of these animals were large, and brown with a grayish tint. All other organs were grossly normal.

The histological examination of the organs gave the following findings:

Vena Cava; Stomach and Intestine; Pancreas; Mesenteric Lymph Nodes. Normal.

Brain. The choroid plexus of the third dog, which received the highest amount of methyl cellulose, consisted of a dense and bulky accumulation of foam cells lined by an intact ependyma. The brains of the other dogs were normal.

Lung. The blood vessels in the interalveolar septa and in the peribronchial tissue were frequently transformed in all 3 dogs into small multicystic structures lined by a syncytium, filled with a colorless, slightly refractive substance, and surrounded by granulation tissue containing large round cells.

Heart. The coronary arteries of the third dog had a thick, foam-cellular intima infiltrating into the media.

Aorta. There was only a minor foam-cellular intimal proliferation involving endothelium and subendothelial tissue in the bulb of the first dog, which showed in a thoracic segment an extensive hyaline and collagenous transformation of the media with marked vascularization (Fig. 3). The intimal foam-cellular cushions in the aortic bulb were more highly developed in the second and third dog, and contained in the second dog a few leukocytes (Fig. 4), while the underlying media showed scattered calcium granules. The thoracic and abdominal seg-

ments of the aortas of these 2 dogs exhibited frequent and marked intimal foam-cellular proliferations which sometimes invaded the intima and also involved the vasa vasorum. The media was often highly edematous or mucoid. A mucoid, small-vesicular zone extended between intima and media. Intimal hyalinization was found occasionally, whereas the media contained larger areas of calcification in both the inner and the hyalinized outer medial zone.

Pulmonary Artery. There was a moderate foam-cellular thickening of the intima of the pulmonary artery near the hilum in the second dog.

Large Elastic Arteries. The large elastic arteries were normal in the first dog, but contained foam-cellular intimal thickenings in the other 2 dogs. The nuclei of these foam cells had sometimes grotesque spider-like chromatin.

Liver. Pericentral congestion and necrosis of liver cells were present in the first dog, while there was increase in size and a foam-cellular transformation of the Kupffer cells and of the interstitial tissue histiocytes in the other 2 dogs. The liver cells were moderately atrophic.

Adrenals. Foam-cellular reticulum cells were found in the medulla of the adrenal of the third dog.

Spleen. The splenic pulp consisted in all 3 animals of large masses of foam cells, none of them invading the lumina of the larger vessels.

Kidney. The cortical tubules were distended and lined by flattened cells. The glomeruli of the second and third dogs showed grape-like cysts. A medium-sized extrarenal artery contained a mushroom-like, hyaline, intimal thickening with underlying thickened but fragmented internal elastic membrane.

Uterus. The uterine arteries of the third dog exhibited a marked foam-cellular proliferation of the intima, often obliterating the lumina (Fig. 5).

Bone Marrow. The sternum contained a hyperemic, immature myeloid tissue.

d. Methyl Cellulose, 100 cps. (Three Dogs)

One dog was injected with 280 cc. of methyl cellulose solution, a second dog received 4645 cc., and a third, 5190 cc. The autopsy showed the lungs to be congested and edematous and containing scattered dark red hemorrhagic areas. The livers in the second and third dogs were enlarged. The spleens of these two animals were about two or three times normal size and each weighed 120 gm. They were grayish red and soft. The renal cortices of these 2 dogs contained numerous wedge-shaped white areas. All other organs were grossly normal.

The histological examination of the organs gave the following findings:

Brain; Thyroid; Large Elastic Arteries; Stomach and Intestine; Pancreas; Lymph Nodes; Bladder. Normal.

Hypophysis. The loose and increased stroma of the predominantly eosinophilic parenchyma contained foam cells in the second and third dogs (Fig. 6).

Parathyroid. Interstitial tissue of the parathyroid glands was increased and contained some foam cells in the third dog.

Lungs. The lungs of the first dog were congested and edematous and showed scattered hemorrhagic areas. The lungs of the second and third dogs contained distended cystic vascular lumina in the peribronchial and interstitial septa, surrounded by multinucleated syncytia and large round cells, fibroblasts and foam cells.

Heart. With the exception of some round cell infiltrations in the myocardium of the second dog, the hearts were normal.

Aorta. The intima of the ascending aorta of the first dog was intact, while the media showed a considerable mucoid imbibition causing the production of small cavities filled with mucoid matter, and a wide separation of the muscle bundles. The media of the thoracic part contained several large hyaline areas. The intima of the aortas of the second and third dogs was transformed into a multilayered foam-cellular coat in which cushion-like foam-cellular thickenings were embedded, which in places extended into the media. These changes were most marked in the bulb. There was an extensive subendothelial calcification in some areas.

Pulmonary Artery. Similar, but not as extensive, intimal and medial lesions, especially also muscular calcifications, were present in the postvalvular portion of the second and third dogs, while the media of the first dog revealed increase of the mucoid content (Fig. 7).

Abdominal Muscular Artery. There was a large area of medial necrosis present, associated with calcification of the elastic fibrils (Fig. 8).

Liver. The liver cells of the first dog were atrophic and the organ was congested. In the second and third dog there was also an atrophy of the liver cells. The cords were pushed far apart by proliferated, foam-cellular Kupffer cells.

Spleen. The splenic pulp of the first dog was hyperemic and cellular, whereas those of the second and third dogs were filled with foam cells which were disintegrating in places.

Adrenals. The adrenals were normal in the first and second dog, but showed foam-cellular reticulo-endothelial cells in the medulla of the third dog.

Kidney. The cortical tubules were distended in all 3 dogs. The glomeruli of the first dog were swollen and rich in nuclei, while the arterioles showed focal hyalinization of the media. The cortices of the second and third contained foci of large mononuclear cells intermingled with a foam-cellular matrix. The glomeruli were transformed into grape-like, cystic formations lined with foam cells or multinucleated syncytia.

Testes. In the second and third dogs there were a few spermatid giant cells in the testicular tubular lumina, which were lined by a generally normal epithelium.

Bone Marrow. The sternum contained a loose myeloid tissue.

e. Methyl Cellulose, 400 cps. (Seven Dogs)

One dog was injected with 260 cc. of methyl cellulose solution, a second and third with 1040 cc., a fourth with 2112 cc., a fifth with 2930 cc., a sixth with 3375 cc., and a seventh with 5720 cc.

The spleens were considerably enlarged in all dogs, including the first one. The organ was grayish pink, soft, and had in some instances an almost liquid, dirty yellow pulp. The weights varied from 230 to 400 gm. The livers were also enlarged, brown-red to brown-gray and displayed a yellowish white network. One liver weighed 1015 gm., while the others were around 500 to 700 gm. The kidneys were large with some retractions in a sometimes gelatinous cortex. The lungs were congested.

Histological examination gave the following findings:

Thyroid and Parathyroid; Stomach; Pancreas; Bladder. Normal.

Brain. The choroid plexuses of dogs 5 and 7 consisted of accumulations of foam cells. Numerous small perivascular glial foci were present in the brain of dog 3, while numerous hemorrhages were found in dog 1.

Hypophysis. Large masses of foam cells separated strands of acidophilic cells in the anterior lobe in dog 7.

Lung. Apart from congestion and the presence of numerous, moderately large round cells with an empty cytoplasm in the interalveolar septa, no pathological lesions were seen in the lungs of dogs 1 to 6. Foam-cellular masses filled the capillaries of dog 7 and formed nodules in the interstitial tissue.

Heart. The myocardium was normal in dogs 1 to 6. There were perivascular foam-cellular foci around the myocardial arteries and arterioles in dog 7. Hyaline thickenings of the media of the myocardial arterioles were present in dogs 3, 6 and 7, associated with foam-cellular intimal proliferations in dogs 6 and 7. Foam cells lined the surface of the aortic valves and the endocardium of the left ventricle in dog 7.

Aorta. The aorta was normal in dogs 1 and 2. The intima of the aortas of the other 5 dogs showed an often multilayered foam-cellular coating and cushion-like thickenings which were most strikingly developed in the aortic bulb and decreased toward the abdominal portion. The media underneath these lesions showed hyaline degeneration sometimes associated with scattered small or large nodules of calcification. Other portions exhibited an increase of the intercellular mucoid matter, which occasionally contained some foam cells.

Pulmonary Artery. The intima consisted of 2 or 3 layers of foam cells covering and invading a hyalinized media, in dogs 4 and 5.

Large Elastic Arteries. Intima showed a foam-cellular coating and small cushions in dogs 4, 5, 6 and 7, while a thickened hyaline intimal cushion was noted in one artery of dog 3.

Muscular Arteries. The intima exhibited occasionally small foam-cellular groups in dog 5.

Vena Cava. The intima consisted of foam cells in dog 6.

Intestine. The mucosa contained foam cells in the interstitial tissue in dogs 6 and 7.

Liver. While the liver of dog 1 showed only scattered foam-cellular granulomatous formations and those of dogs 2 and 3 were merely congested, the livers of dogs 4, 5, 6 and 7 revealed an extensive proliferation of Kupffer cells with transformation into foam cells. The liver cells themselves had in part a vacuolated or foam-cellular appearance. In dog 7 the periportal connective tissue participated in the foam-cellular transformation, which affected also, in part, the endothelium of the hepatic veins.

Spleen. The splenic pulps were always foam-cellular and contained clusters of large multinucleated giant cells arranged in circles. There were large areas of necrosis present. The follicles were atrophic.

Lymph Node. Foam-cellular reticulum cells were found only in dog 7.

Adrenal. Foam-cellular reticulo-endothelial cells in the medulla and glomerulosa of the adrenals were found in dogs 6 and 7. The adrenals were normal in the other dogs.

Kidney. There were a few giant-cellular and foam-cellular granulomas in the interstitial, perivascular tissue of the cortex in dogs 1, 2 and 3. A few glomeruli contained small cystic formations with foam cells. The lesions were much more widespread and severe in dogs 4 to 7, where the glomeruli had a multicystic appearance with endothelial foam cells. The tubules were distended and lined either by flattened cells or by foam cells. Foam-cellular accumulations were seen also in

the interstitial tissue. Foam-cellular proliferations of the intima of intrarenal arteries were present in dog 5 together with medial hyalinizations in the main renal artery.

Testes. Arrest of spermatogenesis existed in dog 2; marked degeneration of the spermatogenic epithelium with the appearance of numerous multinucleated giant cells occurred in dogs 3 and 4.

Uterus and Ovary: Normal in dog 6.

Bone Marrow. The sternum contained an immature myeloid tissue in dogs 1 to 6, while a few foam-cellular reticulum cells were present in an immature myeloid and fatty marrow in dog 7.

f. Methyl Cellulose, 1500 cps. (Three Dogs)

One dog was injected with 990 cc. of methyl cellulose solution, a second with 2330 cc., and a third with 4590 cc. At autopsy the lungs were found to be congested and edematous with hemorrhagic spots; the spleens were enlarged, weighing between 174 and 264 gm.; the livers were brown-red and moderately to markedly enlarged (590 to 920 gm.).

Histological examination showed the following findings:

Brain; Hypophysis; Thyroid and Parathyroid; Large Elastic Arteries; Stomach and Intestine; Pancreas; Lymph Node; Adrenals; Bladder. Normal.

Lung. Numerous distended and multicystic capillaries were found in the interstitial tissue of the lungs. The cysts were lined by a multinucleated syncytium and surrounded by foam cells and large mononuclear cells.

Heart. The myocardium was normal. There were marked foam-cellular proliferations in the intima of the epicardial and myocardial coronary branches in dog 1.

Aorta. The ascending aorta showed a foam-cellular lining of moderate extent and thickness in all 3 dogs. The underlying media was thickened by imbibition of mucoid material. In other parts of the aorta there were some cushion-like foam-cellular intimal thickenings in dogs 1 and 3. The deeper parts of the rather thick cushions in dog 3 were composed of spindle-shaped fibroblasts in radiating arrangement and of hyalin containing scattered foam cells (Fig. 9).

Liver. The liver cells were atrophic. The cords were widely separated by distended sinusoids containing desquamated, swollen, spherical Kupffer cells with foam structure.

Spleen. The pulp of the spleen showed extensive foam-cellular transformation.

Kidney. In the cortex of the kidney the tubules were distended. The

glomeruli were swollen and showed cells with a loose, vacuolar cytoplasm. The edematous interstitial connective tissue contained round cells and multinucleated giant cells.

Testes. The testes were normal in dog 2. The spermatogenic epithelium of the other 2 dogs was markedly degenerated and consisted, in some tubules, of hypertrophic Sertoli's cells only, while in others no epithelial lining was present, but a thickened hyaline capsule surrounded an empty lumen. Some tubules contained deep brown pigmented cells.

Epididymis. The empty ducts of the epididymis were lined by hyperplastic epithelium.

Bone Marrow. The sternum contained fatty myeloid tissue.

g. Methyl Cellulose, 4000 cps. (Three Dogs)

One dog was injected with 2290 cc. of methyl cellulose solution, a second with 3440 cc., and a third with 4930 cc. Autopsies showed that the lungs were edematous and contained some hemorrhagic spots. The livers weighed between 500 and 600 gm., the spleens between 190 and 504 gm. The kidneys were swollen, pale light brown.

Histological examination of the organs gave the following findings:

Brain; Hypophysis; Thyroid and Parathyroid; Pulmonary Artery; Vena Cava; Stomach; Intestine; Pancreas; Bladder. Normal.

Lung. The interalveolar septa were cellular.

Heart. Myocardium and coronary arteries were normal.

Aorta. The aortic intima showed in dogs 1 and 3 a thin foam-cellular proliferation beneath the endothelium. The muscle cells of the media in dog 1 contained fine blue granules or were diffusely incrusted with calcium salts, particularly beneath the intima. There was a more massive foam-cellular intimal proliferation in the ascending aorta of dog 2, associated with imbibition of mucoid material in the media. The other aortic segments were normal.

Large Elastic Arteries. Some scattered small, foam-cellular, intimal proliferations were found, but the majority of the large arteries were normal.

Liver. In the 3 dogs there was an extensive destruction of the liver parenchyma leaving often only a reticular framework and proliferated foam-cellular Kupffer cells.

Spleen. There was extensive necrosis in a foam-cellular pulp in the spleens of dogs 1 and 2, while numerous multinucleated giant cells, arranged in clusters, and massive accumulations of foam cells were present in dog 3.

Adrenals. Some foam-cellular reticulum cells were present in the medulla of the adrenals in dog 2.

Lymph Nodes. The mesenteric nodes of dog 2 showed numerous multinucleated giant cells, mainly in the peripheral sinuses.

Kidney. The renal cortical tubules were distended and lined by a flattened epithelium. The glomeruli were converted into multicystic grape-like formations with foam-cellular endothelium. There were localized round cell infiltrations in the interstitial tissue, associated with foam cells in dog 3.

Uterus. Normal in dog 3.

Testes. The spermatogenic epithelium was highly atrophic and the Sertoli's cells were hypertrophic in dog 2.

Bone Marrow. The sternum contained a dense mature myeloid tissue.

Foam-cellular transformation of the supporting tissue of the choroid plexus of the brain was found only in dogs injected with large amounts of methyl celluloses of 50 and 400 cps., while similar changes in the anterior lobe of the hypophysis were seen in animals treated with large doses of methyl celluloses of 100 and 400 cps. The presence of multicystic, distended pulmonary capillaries and precapillary vessels, lined with multinuclear syncytia and surrounded by large mononuclear cells and foam cells, was noted only in dogs into which methyl celluloses of 50, 100, 400 and 1500 cps. had been introduced. Dogs receiving the two methyl celluloses with lower viscosities and the one with a higher viscosity were free from such lesions. The dogs injected with methyl cellulose of 15 cps. did not exhibit degenerative and foam-cellular vascular reactions, such as were seen in animals treated with methyl cellulose of the other types. Although their occurrence and extent showed a dependence upon the amount of methyl cellulose solution introduced, the foam-cellular intimal response as well as the hyaline and calcifying medial changes were comparatively mild in the dogs which received methyl cellulose of 4000 cps. The livers of dogs treated with methyl celluloses of 15, 25 and 50 cps. showed evidence of direct liver necrosis in the absence of extensive storage of methyl cellulose in swollen, proliferated, foam-cellular Kupffer cells. In dogs injected with the higher molecular methyl celluloses such degenerative hepatic lesions were usually secondary to extensive proliferative reactions in the Kupffer cells, associated with the storage of methyl cellulose in these elements. This type of hepatic parenchymatous destruction was marked in the dogs which received methyl cellulose of 4000 cps. The presence of methyl cellulose within liver cells was noted only in dogs injected with methyl cellulose of 400 cps. In all other dogs the liver parenchyma appeared to be free from methyl cellulose, but sometimes contained fat. The retention of methyl cellulose in the reticulum and reticulo-endo-

thelial cells of the spleen and the transformation of these cells into foam cells and multinucleated giant cells was found in dogs of all series. A similar uniformity was noted in the retention and deposition of methyl cellulose in the glomeruli of the kidneys, in the degenerative changes in the spermatogenic epithelium of the testes, and in respect to the absence of foam cells in the bone marrow.

Comment

From the data recorded it becomes evident that the various intravenously injected methyl celluloses elicit, regardless of their molecular weights, changes in the blood characteristic of the hematologic macromolecular syndrome (primary leukopenia, secondary leukocytosis, anemia, increased sedimentation), as well as organic lesions (foam-cellular masses in liver, spleen, adrenal, kidney), typical of the storage of macromolecular colloidal matter, such as glycogen, lipoids, polyvinyl alcohol, silica, pectin, gum arabic, etc., *i.e.*, substances which form emulsions with hemoplasmatic and cytoplasmatic protein solutions.[8] However, this reactive pattern was not uniformly developed with all methyl celluloses. If cognizance is taken of those deviations which are attributable to the differences in the amount of material injected, such as the appearance of foam cells in the choroid plexus, in the anterior lobe of the hypophysis, and in the adrenals, there remain discrepancies which seem to be causally related to differences in the physicochemical properties of the injected agents.

The shorter duration of the leukopenia after a single injection of methyl celluloses of 15 and 25 cps., the milder decrease in the number of erythrocytes and in hemoglobin and volume of packed blood cells, the delayed appearance and shorter duration of the acceleration in erythrocytic sedimentation, and the more rapid transitory elevation of the plasma viscosity, as compared to the findings after the introduction of methyl celluloses of higher molecular weights, obviously reflect the influence of the relative molecular size upon the intensity and duration of such acute colloidal reactions. This is particularly true, since a comparatively larger amount of the low molecular methyl celluloses was contained in the injected dose of 40 cc. than was present in solutions of methyl celluloses of higher molecular weight. This conclusion is supported by observations made by Bucher [13] in connection with the intravenous injection of colloidal solutions of glycogen into rabbits. This investigator found that the severity and duration of the leukopenic reaction was dependent upon the quantity as well as upon the molecular size of the glycogen injected.

However, this relationship concerning the influence of the molecular

size upon the severity and type of reaction was not consistent in respect to the plasmatic viscosity reactions elicited by methyl celluloses of higher molecular weight, as this response was very mild and of very short duration for treatment with both single and repeated doses. The relatively very small absolute amounts used of methyl celluloses of 1500 and 4000 cps. may have militated against any marked and prolonged elevation of the plasma viscosity. On the other hand, it may be possible that the very long-chained molecules of methyl celluloses of 1500 and 4000 cps., when introduced into the blood, do not preserve their rod-shaped form and their directed arrangement responsible for the high viscosity of their aqueous solution, but follow the example of the protein molecules and curl up into globules, thereby causing a marked diminution of the viscous properties. This suggestion is based on a claim of Lepeschkin,[14] who recently reported the occurrence of such changes for the filamentary gelatin molecules when a gelatin solution is heated from just above the jellying point to higher temperatures at which the gelatin is perfectly liquid and much less viscous. An additional causal relation between molecular size and plasmatic viscosity appears to be reflected in the rapid decrease of plasma viscosity after cessation of treatment in animals injected with methyl celluloses of 50 and 100 cps., in contrast to the long-continued elevation of this factor in dogs receiving methyl cellulose of 400 cps.

A similar parallelism between the action of the two low molecular methyl celluloses and the very high molecular cellulose exists in regard to the absence of intracapillary retention cysts of the lungs after their injection. Such lesions were regularly found with the other types. Primary or secondary differences in molecular size and configuration among the various methyl celluloses seem to offer the most plausible explanation for the discrepancies between these anatomical reactions, the development of which depends to a definite degree upon the width of the pulmonary capillaries.

It is noteworthy in this connection that retention of methyl cellulose within the glomerular filter, associated with foam-cellular and proliferative reactions of the endothelium, was found with methyl celluloses of all types regardless of their molecular weights. This is remarkable since three of these substances have a molecular weight below 65,000, which is supposed to represent the critical level for glomerular filtration of macromolecular substances. The observation shows that molecular size determined for the dry material is evidently not the only factor in determining filtrability, but that the degree of molecular hydration plays an important rôle in this respect. Similar results were obtained by Bott and Richards [15] in tests of the permeability of the

glomerular membrane of the amphibian kidney for serum albumin of duck's and hen's eggs, lactoglobulin, zinc insulin, horse serum albumin, tuberculin protein, salmine, and inulin, as only inulin, with a molecular weight of about 6000, filtered through completely. All other agents studied were retained to some degree.

It must remain uncertain whether the molecular weight and perhaps also the molecular configuration of methyl cellulose of 4000 cps. may be responsible for the unusually severe anemia and the extraordinary hyperleukocytosis observed in two of the three injected dogs. Also remarkable is the relatively slight severity and extent of the foam-cellular intimal, and hyalinizing and calcifying medial, lesions of the aorta and large elastic arteries in dogs treated intensely with methyl cellulose of 4000 cps., as compared to the vascular changes in animals treated with methyl celluloses of lower molecular size.

Other striking relations between molecular size of methyl cellulose and biological effect were found in its storage in the liver and in the coagulation time of the blood. Whereas the Kupffer cells and also, after intensive treatment, the histiocytes of the periportal connective tissue of dogs injected with any of the methyl celluloses exhibited a marked foam-cellular transformation and proliferation associated with atrophy of the liver cells and, with the high molecular methyl cellulose, extensive destruction of liver cells, it was only in dogs receiving methyl cellulose of 400 cps. that the liver cells also retained methyl cellulose. It is significant that dogs of this series only displayed a considerable lengthening of the clotting time, while the dogs of all other series showed no abnormal fluctuations of this factor. The presence of hepatic necrosis did not seem to influence the clotting time.

It may be added that the repeated intravenous introduction of methyl cellulose solutions of the different types resulted in the production of vascular lesions characterized by foam-cellular proliferations of the endothelial and intimal cells, sometimes associated with fibroblastic and hyaline intimal thickenings, increased mucoid imbibition of the media which sometimes resulted in the formation of small, irregular, subintimal cavities filled with mucoid material, and with hyalinization and calcification of the media. These various reactions, which increased in extent and degree with the intensity of the treatment, were found in both elastic and muscular arteries but were more marked and more frequent in the former. Although they frequently occurred together, it was not unusual for intimal or medial changes to be seen alone. The vasa vasorum of the aorta sometimes participated in the foam-cellular reactions. It is noteworthy that occasionally extensive areas of medial calcification without intimal lesions or with foam-cellular intimal pro-

liferation were encountered in medium-sized abdominal branches of the aorta. These observations strongly suggest that also in the development of the degenerative intimal and medial lesions in man, particularly those of the Mönckeberg type, common causal factors are active, and that medial calcinosis of the peripheral arteries does not represent a special type of vascular disease which must be distinguished etiologically from ordinary atherosclerosis.[16-19]

The high frequency of severe testicular atrophy in the dogs of this experiment supports the conception advanced in previous publications that anoxemia is the fundamental common causal factor in the production of degenerative vascular disease, as testicular degenerations are a phenomenon frequently met with in anoxemic states (chronic mountain sickness, lead poisoning, nitrate poisoning, nicotine poisoning, carbon disulfide poisoning, carbon monoxide poisoning, etc.) [17, 20]

Conclusions

1. The intravenous injection of single and repeated doses of seven types of methyl cellulose ranging in molecular weight from 32,200 to 143,000 results in hematic and organic reactions which differ among themselves in some respects, depending upon the molecular weights.

2. Methyl celluloses of low molecular weight cause a shorter duration of a transitory leukopenia, a milder decrease in the number of erythrocytes, a shorter acceleration of erythrocytic sedimentation and a shorter elevation of the plasmatic viscosity than those elicited by methyl celluloses of higher molecular weights.

3. Methyl celluloses of high molecular weight form an exception to the rule in regard to the increase in plasmatic viscosity, as they do not elicit an appreciable elevation of this factor even after the introduction of large amounts.

4. A similar parallelism between molecular weight and anatomical lesions is represented by the fact that the two methyl celluloses of lower molecular weight do not produce intracapillary pulmonary retention cysts, as do all other methyl celluloses with the exception of the methyl cellulose of highest molecular weight.

5. All methyl celluloses were retained at least in part by the glomerular filtration membrane, indicating that here the degree of hydration plays an important rôle by influencing molecular size.

6. Lengthening of the clotting time was found only with methyl cellulose of 400 cps., which has about the same molecular weight as serum albumin and enters the liver cells, which do not store the other methyl celluloses.

7. The degree and distribution of atheromatous intimal, and hyalin-

izing and calcifying medial, lesions in the elastic and muscular arteries increased with the intensity and duration of treatment, but varied somewhat with the type of methyl cellulose injected, being least developed with the very high molecular type.

8. The frequent occurrence in muscular arteries of medial calcification unrelated to atheromatous changes indicates that the same causative mechanism is active in the production of both intimal atheromatosis and medial calcinosis.

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[*Illustrations follow*]

DESCRIPTION OF PLATES

PLATE 141

FIG. 1. Myocardial necrosis and calcification with proliferation of fibroblasts and with infiltration of leukocytes and mononuclear cells. An artery shows considerable proliferation of intimal foam cells. Hematoxylin and eosin stain. × 360.

FIG. 2. Large elastic artery with mild foam-cellular proliferation of the intima and a large focus of necrosis and calcification in the middle media. Hematoxylin and eosin stain. × 360.

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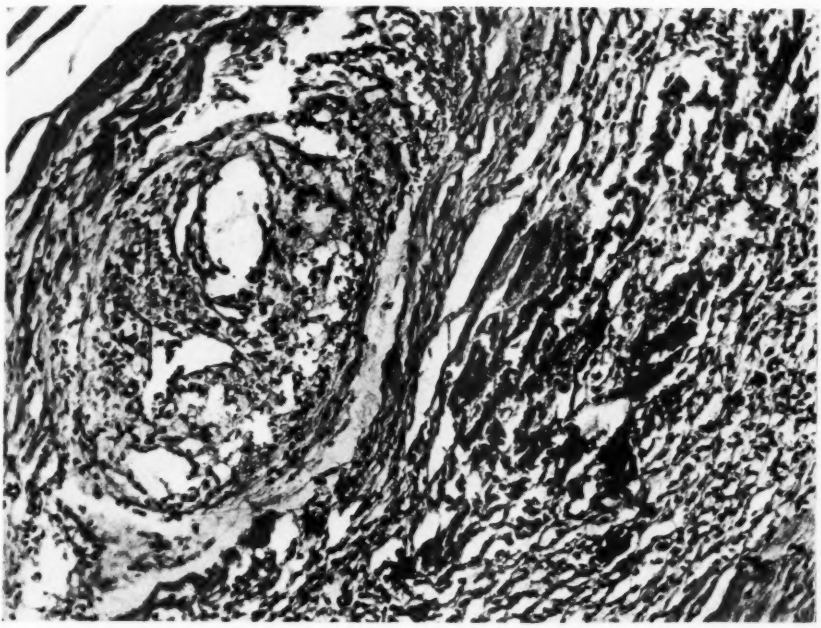

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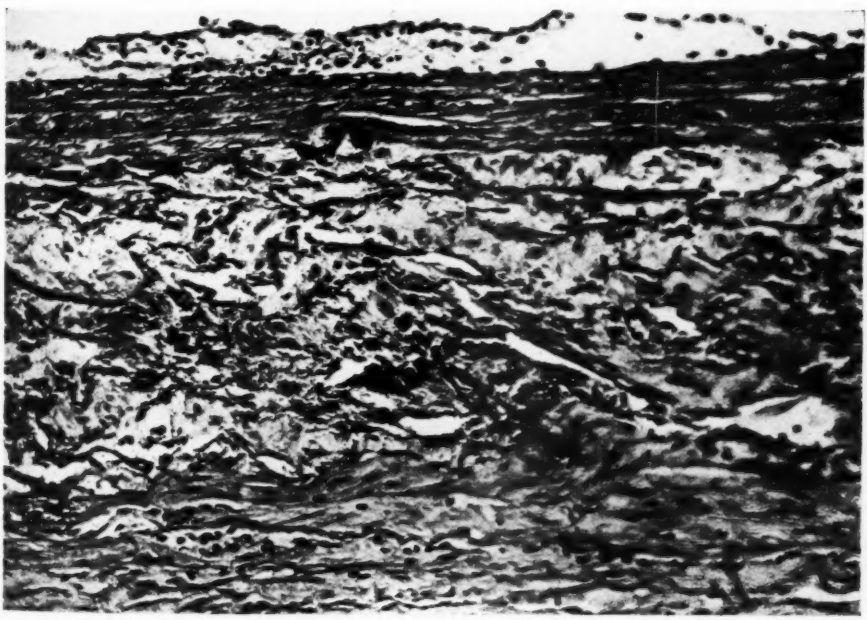


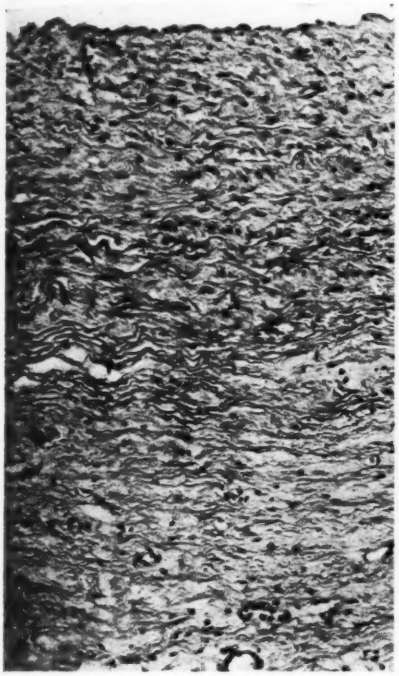
Hueper Intravenous Injections of Methyl Celluloses

PLATE 142

FIG. 3. Extensive area of fibrosis and hyalinization in the outer half of the aortic media (lower part of figure) with marked capillary proliferation and mild infiltration with lymphocytes. Hematoxylin and eosin stain. × 310.

FIG. 4. Cushion-like, cellular intimal thickening of the aorta with invasion of scattered leukocytes into a highly mucoid media. Hematoxylin and eosin stain. × 310.

FIG. 5. Uterine arteries with extensive foam-cellular intimal proliferation almost occluding the lumina. Hematoxylin and eosin stain. × 360.



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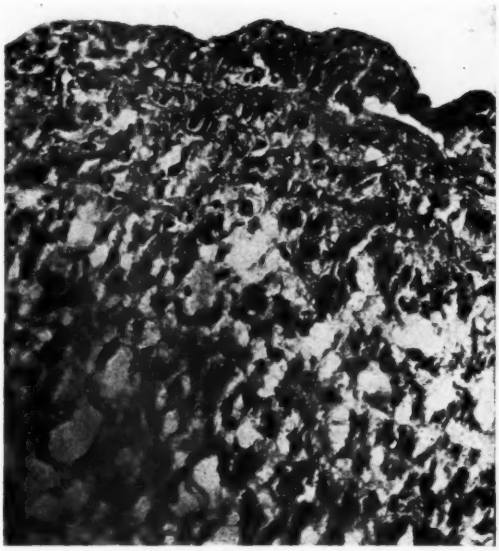

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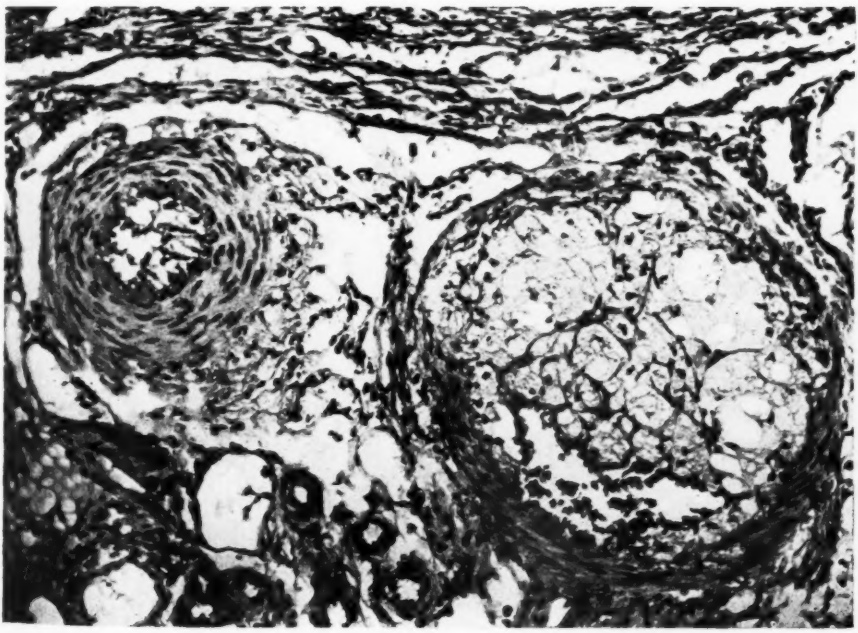

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Hueper

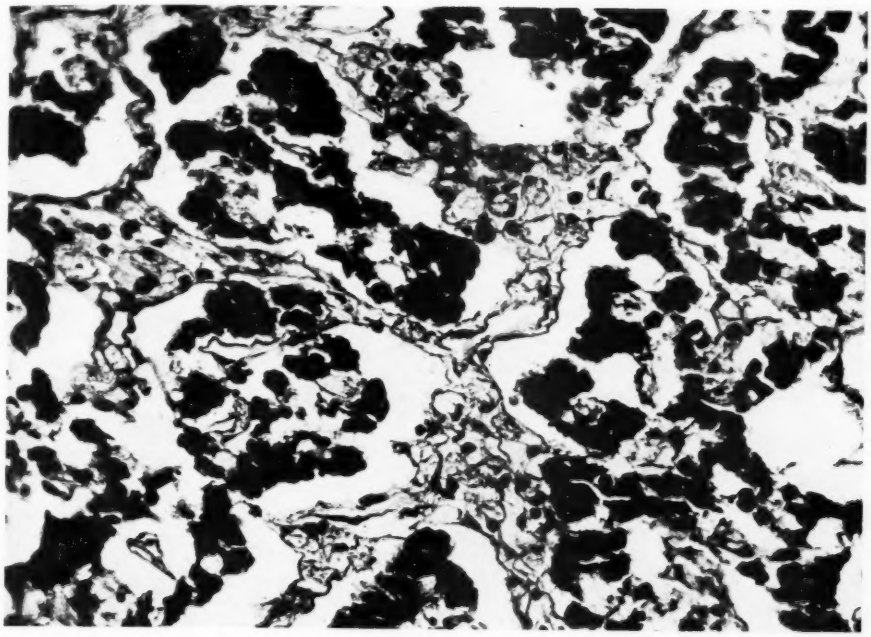
Intravenous Injections of Methyl Celluloses

PLATE 143

FIG. 6. Anterior lobe of the hypophysis with foam-cellular interstitial tissue. Hematoxylin and eosin stain. × 660.

FIG. 7. Pulmonary artery with foam-cellular thickening of the intima and with hyalinization and calcification of the media. Hematoxylin and eosin stain. × 360.

6



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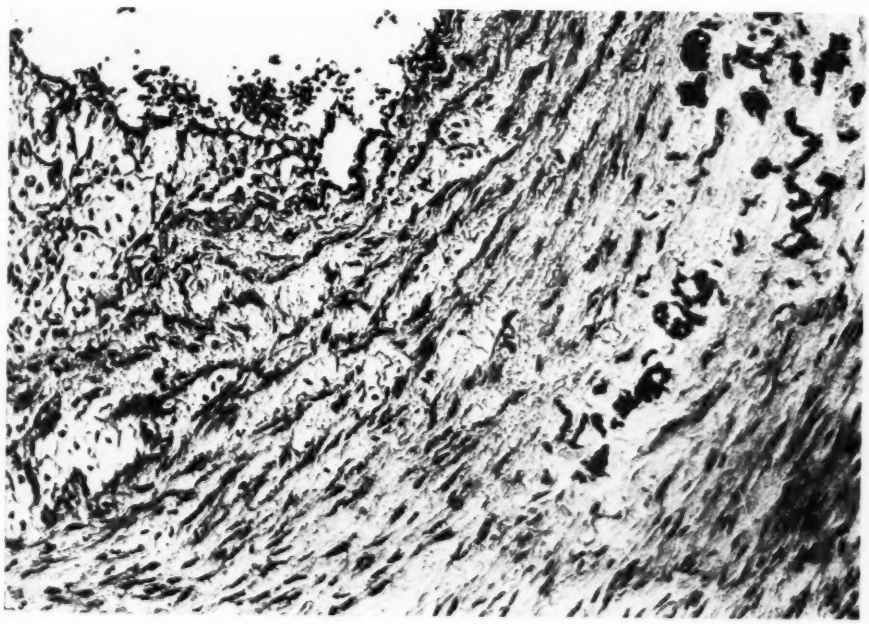


Hueper

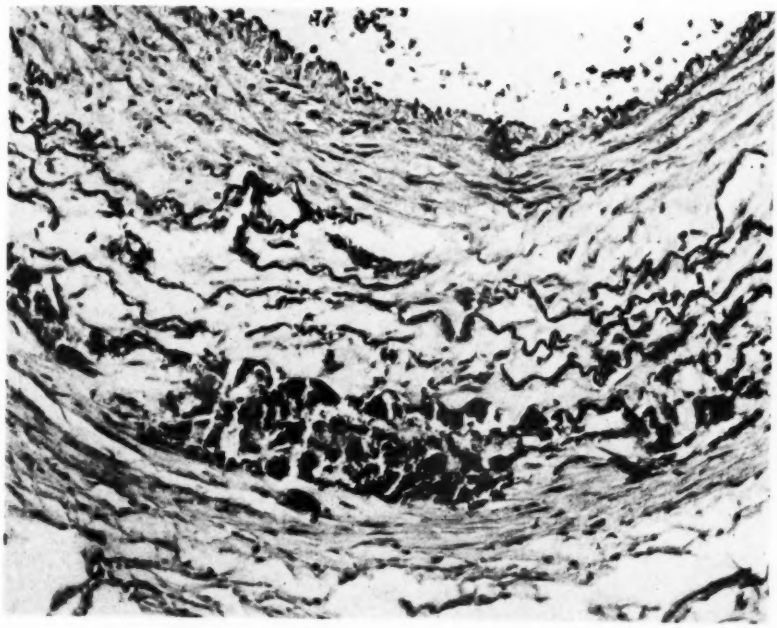
Intravenous Injections of Methyl Celluloses

PLATE 144

FIG. 8. Large muscular artery with extensive medial necrosis, and calcification of the necrotic matter and of the elastic fibrils. Hematoxylin and eosin stain. × 360.

FIG. 9. Intimal thickening of the aorta consisting of a foam-cellular inner layer and a deeper, partly hyaline, partly fibroblastic layer covering a highly mucoid media. Hematoxylin and eosin stain. × 360.

8



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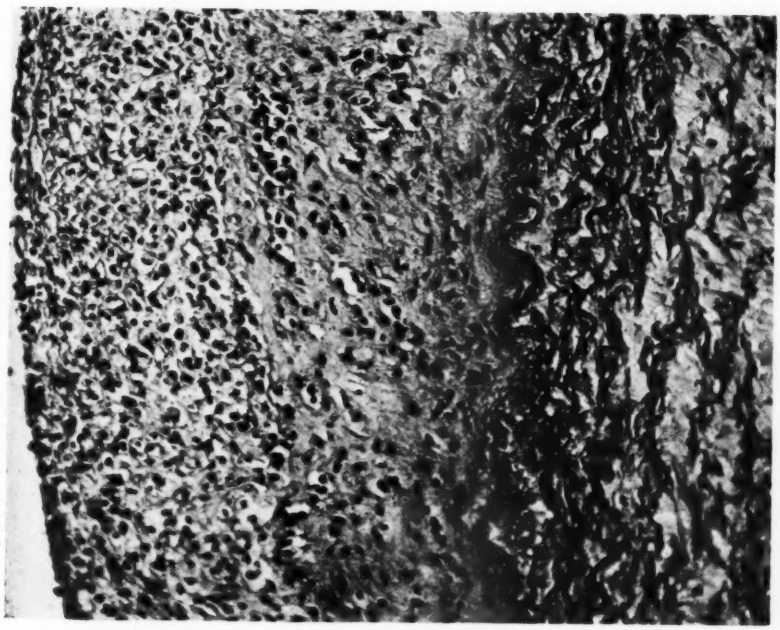

Hueper Intravenous Injections of Methyl Celluloses

SPONTANEOUS AND TRANSPLANTED RETICULUM CELL SARCOMAS IN WISTAR RATS*

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Within the past year, 14 spontaneous sarcomas of lymphatic tissue were found in a group of rats comprising 1000 Wistar albinos and 185 gray Norways, maintained for studies in problems of aging in The Wistar Institute animal colony. Five of the tumor-bearing rats were of the gray Norway strain (2 males and 3 females, ranging in age from 370 to 670 days), and 9 were of the Wistar albino strain (3 males and 6 females, ranging in age from 331 to 555 days). Three of the albino rats had been splenectomized. Approximately 67 per cent of the rats of the entire group were as old or older than the youngest tumor-bearing member. The neoplasms were diagnosed as reticulum cell sarcomas, and were successfully transplanted.

Gross Appearance of Spontaneous Tumors

Ascites was always present; more than 40 cc. of cloudy, bloody fluid could be withdrawn from the abdominal cavity. The largest tumors occurred in the omentum and in the mesentery at the ileocolic junction. They consisted of small semitranslucent bodies or of larger, irregular, lobulated masses, which were partially hemorrhagic. A similar growth was found at the site of the thymus in all but four rats. In advanced stages, the mediastinal, renal, pelvic and retroperitoneal lymph nodes were involved. The axillary, inguinal and cervical lymph nodes were invaded in one albino rat.

In one of the gray Norway rats multiple tumorous growths studded the diaphragm and abdominal walls. Microscopical sections of the intestine showed evidence of invasion of the muscles but not of the mucosa (Fig. 1). In the majority of the tumor-bearing rats, part of the pancreas was replaced by neoplastic tissue, and in three instances the liver was infiltrated. Other organs appeared to be normal, and the neoplasm was not found in the spleen, kidneys, adrenals, lungs, or gonads in microscopical preparations.

Cells of Spontaneous Tumors

The neoplasm was composed of closely packed, large mononuclear cells, usually round or oval in shape, and several times the diameter of a normal lymphocyte. The nuclei were sharply outlined, and were for

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the most part vesicular, containing one or two prominent nucleoli. Mitoses were common. The amount of finely granular cytoplasm varied in each cell, but tended to be scanty. A microscopical section through one of the tumors is shown in Figure 2.

In silver-stained preparations, for which we are indebted to the Army Medical Museum, "a delicate reticulum is seen to enmesh the cells in some parts of the tumor; in other areas the growth is solidly cellular and lacks stroma of any kind (Figs. 5 and 6). This variation is interpreted as signifying that in the older parts of the tumor reticulum has been formed, while the cells of rapidly growing parts have not as yet elaborated reticulum fibrils." *

Transplantations

The first transplantation of a spontaneous tumor from an albino (444 days old) into rats of the same strain failed to grow in either of the recipients. The second transplanted spontaneous tumor (also from an albino, 531 days old) has been carried successfully through thirteen passages in Wistar albino rats. Thirty-one rats received implants, some subcutaneously and some intraperitoneally, and the tumor grew in 29 of them. The recipient rats ranged in age from 16 to 81 days when implanted. They were not related to the original donor, but the majority of them were bred from the littermates of rats that had proved to be susceptible in early passages. Our results show that the younger the recipient the more readily the tumor grew. Regardless of the site of implantation, the resulting neoplasm usually resembled the spontaneous tumors.

In some of the rats receiving grafts the tumor grew subcutaneously at the point of entrance of the trochar. In one of these the neoplasm remained localized. After 64 days it measured 50 by 42 by 25 mm. Intraperitoneal growth, as seen in the other hosts, was characterized by ascites, rapid spread and enlargement of the tumor, and early death. A rat 81 days old when inoculated died after 73 days, but in younger hosts abdominal tumors caused death in 20 days on the average.

Cells from the body fluids and pieces of the sarcomas engrafted into the abdominal cavity of young rats produced discrete, often hemorrhagic nodules, principally in the omentum but also in the mesentery, in the form of beads paralleling the intestine. If the host survived long enough, the smaller growths coalesced into masses characteristic of the spontaneous tumors. Figure 7 illustrates an early stage in the fusion of small tumors, in which the outlines of the individual nodules are discernible.

*Quoted from a letter from Col. J. E. Ash in regard to U. S. Army Medical Museum Accessions nos. 91454 and 91258 a and b.

In rats receiving grafts, usually a greater number of abdominal lymph nodes were affected, and more extensively, than in rats in which the tumor occurred spontaneously. Growths of the malignant cells occurred in the thoracic cavity of the majority of the treated rats. Invasion of the liver and the pancreas (Fig. 3) was observed, but not of spleen, kidneys, adrenals, lungs, or gonads. However, inoculation of a fine suspension of tumor cells directly into spleen and kidney produced typical growths within these organs.

Microscopically the cells of tumors that arose from grafts appeared to be identical with those of the spontaneously occurring sarcomas (Fig. 4). In the more recently developed masses reticulum was lacking, but in the older parts of the tumor a well developed reticulum enmeshed the cells (Figs. 5 and 6).

Malignant Cells Found in Body Fluids

Routine cell counts of peripheral blood from nine inoculated rats and four with spontaneous neoplasms were made before the animals were sacrificed. In one of the former, the red cell count was 2,370,000 cells per cmm.; and in one of the latter, 4,680,000 cells per cmm. Erythrocyte counts in the other rats averaged 6,818,000 per cmm. White cells ranged from 4,950 to 21,900 per cmm., the higher number occurring in an older rat with only subcutaneous tumors; the average was 9,330 per cmm. The differential blood counts were for the most part within the usually accepted normal ranges for rats; [1] averages were: neutrophils, 26.8 per cent; lymphocytes, 72.2 per cent; monocytes, 0.5 per cent; eosinophils, 0.4 per cent; basophils, 0.1 per cent. Large lymphocytes were frequently noted. One malignant cell, conspicuous among the smaller normal cells, was recognized in two spreads. With Wright's stain the large round nuclei were lavendar and finely granular, and the narrow rim of cytoplasm a deep blue. A section of a buffy coat of centrifugalized blood presented an aggregate of malignant cells that closely resembled a formed tumor.

Examination of ascitic fluid from tumor-bearing rats revealed an abundance of malignant cells, indistinguishable from those seen in blood smears, many of which were in mitosis. In a spread of ascitic fluid the size of the enlarged cells was three to five times that of normal lymphocytes. Numerous mast cells and eosinophils also were present.

Intraperitoneal injections of the following substances were made from tumor-bearing hosts into healthy young albinos: blood plasma (into five rats), blood cells (four rats), whole blood (five rats), cells from ascitic fluid (three rats), and ascitic supernatant fluid (into four rats). Typical sarcomas appeared after inoculation of the cells but not following injections of cell-free material. The failure to produce tumors

by the injection of plasma and cell-free ascitic fluid indicates that the causative factor of this tumor is not a virus.

Discussion

A tumor, occurring spontaneously in 14 of the rats of the Wistar colony devoted to study of aging, has been identified as a reticulum cell sarcoma. Grossly, it conforms to McEuen's[2] description of spontaneous lymphosarcomata that occurred in a colony of hooded rats. It resembles the lymphosarcomata studied by Curtis and Dunning[3] except that in our rats perforation of the gut was not found and malignant growths frequently occurred in the thoracic cavity. The tumor seems to resemble closely the reticulum cell sarcoma described by Jenney,[4] although we have not found the invasion of spleen, kidneys, or lungs, or the leukocytosis following implantation, that she noted.

The location of the early sarcomas in the mesenteries suggests that some element in the absorbed food might induce tumor formation, particularly as the rats used by McEuen[2] and by Jenney[4] were fed the same diet as our animals. However, in another group of gray Norway and albino rats, raised in the Wistar colony under the same conditions and on the same diet as those reported here, reticulum cell sarcomas were not observed, although routine autopsies were not performed.

Nelson and Morris,[5] reporting a reticulum cell lymphosarcoma in rats which differed from ours in the location of the original growth, ruled out pulmonary infection as an apparent cause of the neoplasm. In the rats of Curtis and Dunning,[3] ulceration of the gut was common but did not invariably accompany the neoplasm. We, too, found no indication of a relation between infection, or any other disorder, and the spontaneous reticulum cell sarcomas. Pathological conditions other than the neoplasms themselves were not observed. Perforation or ulceration of the gut was not found, and necrosis in tumors was not constant. Tissue cultures of the sarcomas, made at The Wistar Institute, were free of bacteria.

Summary

1. A spontaneous reticulum cell sarcoma was found in three female and two male gray Norway rats, 370 to 670 days old, and in six female and three male Wistar albino rats, 331 to 555 days old, in a group of over 1000 animals.

2. The neoplasm occurred in the mesentery and in the omentum, and was found frequently in the thoracic cavity and occasionally in abdominal lymph nodes and liver, but not in other organs. Ascites accompanied the growth of the tumor.

3. The spontaneous tumor in one Wistar albino rat, 531 days of age,

was successfully transplanted into 2 young rats of the same strain. The transplanted tumor was carried through thirteen successive passages, and tumors resembling the spontaneous sarcoma developed in 29 of the 31 rats engrafted during these passages.

4. Subcutaneous implants grew slowly and tended to remain localized. Grafts in the abdominal cavity spread quickly and caused death of the hosts in an average of 20 days.

5. The cells of spontaneous and implanted tumors were indistinguishable. Reticulum was found only in older growths of both the spontaneous and the engrafted sarcomas.

6. Malignant cells were identified in the blood and ascitic fluid of tumor-bearing rats. Intraperitoneal injection into healthy young albino rats of the centrifugalized cells from these fluids produced typical sarcomas; injection of plasma and cell-free ascitic fluid did not produce tumors.

We wish to express our sincere appreciation to Dr. Margaret Reed Lewis for her aid and direction; and to Dr. George W. Corner of the Carnegie Institute of Washington for help with some of the photographs. We also wish to thank Col. J. E. Ash, Curator, and Lt. Col. Balduin Lucké of the Army Medical Museum, Institute of Pathology, for their interest and cooperation in the identification of the tumors, and for their kindness in furnishing two of the photographs used in this paper.

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[*Illustrations follow*]

DESCRIPTION OF PLATES

PLATE 145

FIG. 1. Muscle wall of intestine invaded by cells from an adjacent tumor in the mesentery. × 189.

FIG. 2. Section of a spontaneous reticulum cell sarcoma. × 472.

FIG. 3. Invasion of pancreatic tissue by sarcoma cells. × 472.

FIG. 4. Section of reticulum cell sarcoma from a recipient rat. × 472.

FIG. 5. Silver-stained section of young tumor showing small amount of reticulum. × 212. (U. S. Army Medical Museum Neg. no. 76210. Acc. no. 91258a.)

FIG. 6. Heavily stained, abundant reticulum in older tumor. × 212. (U. S. Army Medical Museum Neg. no. 76209. Acc. no. 91258a.)

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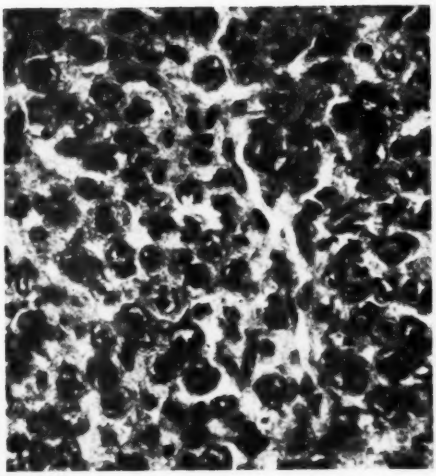

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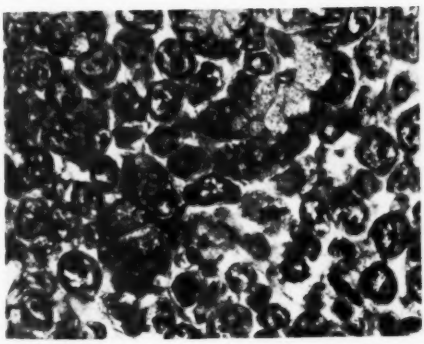

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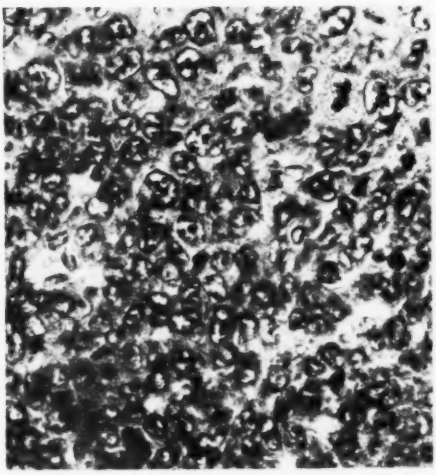

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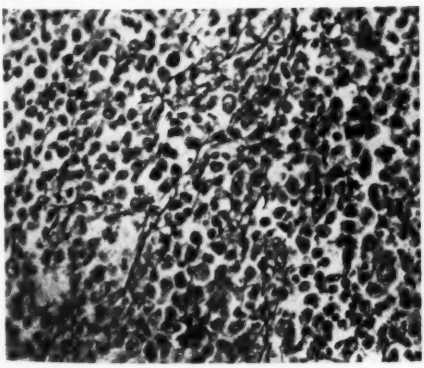

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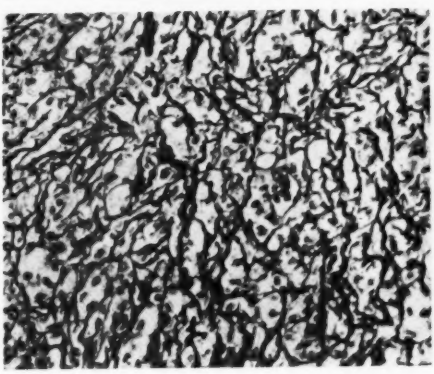

6

Farris and Yeakel

Reticulum Cell Sarcomas in Wistar Rats

PLATE 146

FIG. 7. Widespread development of reticulum cell sarcoma in a recipient rat.

(a) Subcutaneous tumor at the site of inoculation.
(b) Tumor in the thoracic cavity.
(c) Spleen.
(d) Tumor in the omentum.
(e) Nodules in the mesentery by the small intestine.
(f) Growths in the connective tissue by the gonads.

Farris and Yeakel Reticulum Cell Sarcomas in Wistar Rats

NONSUPPURATIVE NODULAR PANNICULITIS (WEBER-CHRISTIAN'S DISEASE)*

DAVID M. SPAIN, M.D., and JOSEPH M. FOLEY, M.D.
(From the Laboratories of Pathology, Bellevue Hospital, New York, N.Y.)

Weber-Christian's disease is a clinicopathological entity characterized by recurring episodes of fever and the occurrence of numerous, painful, slightly tender, subcutaneous nodules. These nodules consist of nonsuppurative areas of inflammation and necrosis in the subcutaneous fat. In only 2 of the 24 cases reported in the literature was death apparently due to the disease. In one of these cases a necropsy was performed and reported by Kritzler.[1] The literature on the reported cases is adequately reviewed by Bailey.[2] The object of this report is to present an additional case with necropsy. The patient reported upon here, although dying in uremia as a result of chronic glomerulonephritis, was in the active phase of nonsuppurative nodular panniculitis.

REPORT OF CASE

P. F., an unmarried Irish male, 51 years old, was admitted to the First Medical Service at Bellevue Hospital on July 12, 1941. The chief complaint consisted of generalized aching pain involving all of his muscles. This was of 1 week's duration. The patient had a previous history of three admissions for alcoholism to the Psychiatric Division of this hospital. The last admission was 5 years prior to the present illness. Three days before the present admission he also complained of passing black stools with flecks of red blood and the following day there were two episodes of epistaxis. Due to the patient's mental state, reliable personal and family history could not be obtained.

On admission the temperature was 98.6° F.; pulse, 80; respirations, 20; blood pressure, 140/100 mm. of Hg. The patient was drowsy, slightly confused, but cooperative. Speech was thick and slurred. No abnormalities of the skin were noted at this time other than a few areas of ecchymosis over the right tibia. Except for some abdominal tenderness in the right upper quadrant, there were no other significant findings on physical examination.

Two days after admission several small subcutaneous, pea-sized nodules were noted in both arms and legs. These were slightly tender and freely movable beneath the skin. The overlying skin showed no changes. At the same time the patient began to have an irregular fever ranging between 99° and 104° F.

Laboratory examinations at this time revealed the following: red blood cells, 3,500,000; hemoglobin, 8.5 gm.; white blood cells, 8,500; polymorphonuclear leukocytes, 85 per cent; lymphocytes, 15 per cent. Platelets were within normal range. No eosinophils were seen. Specific gravity of the urine, 1.010 and 1.016; albuminuria, 1 to 2 plus and occasional granular casts. Blood nonprotein nitrogen, 182 to 275 mg. Phosphatase, 4.3 Bodansky units. Albumin-globulin ratio, 4.1/2.0. Carbon dioxide combining power varied between 20 and 52. Cephalin-flocculation test, 2 plus. A stool contained bright red blood. Wassermann reactions on blood and spinal fluids were negative. Roentgenograms of thorax and abdomen revealed no abnormal-

* Received for publication, September 21, 1943.

ities. Serum agglutination tests were negative for typhoid and paratyphoid bacilli and *Brucella melitensis*. Skin tests with trichinella antigen were also negative.

One of the subcutaneous nodules was removed with the surrounding adipose tissue. The specimen measured 2 by 1 by 1.5 cm. In the central portion of this mass was a somewhat firm, palpable nodule that on section appeared yellowish gray. Radiating streaks of this yellowish gray material extended from the central area into the adjacent fat. Histologically the nodule was an area of fat necrosis with a surrounding infiltration of lymphocytes, occasional polymorphonuclear leukocytes and numerous fat-laden macrophages. There was a moderate increase of fibrous tissue immediately adjacent to this lesion. Scarlet R stain showed material within the phagocytic cells to be fat. A diagnosis of nonsuppurative nodular panniculitis (Weber-Christian's disease) was made.

The patient's condition became steadily worse. He eventually went into uremic coma and died 10 days after admission. During this period, however, some of the nodules first noted seemed to disappear or, at least, to become reduced in size.

Necropsy Findings

The subcutaneous fat was examined carefully and several of the nodules previously described were found. Similar nodules were found also in the mesenteric, omental and pretracheal fat deposits. These nodules were 2 to 5 mm. in diameter and varied from dull gray to yellowish gray. The kidneys were reduced in size, had a granular surface and the cortex was reduced in width with striations blurred and poor differentiation from the medulla. The liver was of the usual size, somewhat flabby and yellowish in appearance.

Histological Examination. The nodules in the fat were of varying appearance. The earliest and smallest lesions consisted merely of small accumulations of fat-laden macrophages (Fig. 1). Lesions somewhat larger had small central areas of necrosis (Fig. 2), about which were lymphocytes, polymorphonuclear leukocytes and numerous fat-laden macrophages (Fig. 3). In the obviously older nodules the necrotic material was decreased and the inflammatory cells had been replaced by fibrous tissue (Fig. 4).

The alterations in the kidney were characteristic of the end stage of chronic glomerulonephritis. Numerous fat droplets were contained within the liver cells. Small areas of fat necrosis were present about the pancreas. These had the appearance characteristic of pancreatic fat necroses and did not resemble the necrotic nodules in the subcutaneous tissue.

The final diagnosis was chronic glomerulonephritis with uremia, nonsuppurative nodular panniculitis, pancreatic fat necrosis and fatty change of the liver.

Comment

In the case reported by Kritzler,[1] the characteristic lesions were limited to the subcutaneous fat. However, fat emboli were found in the lungs and there was widespread acute necrosis of the liver and spleen.

The liver showed fatty change. In the case presented in this report characteristic necrotic areas were found not only in the subcutaneous fat but in the mesenteric, omental and pretracheal fat. There were no fat emboli in the lungs. Areas of necrosis in the liver and spleen were not observed. However, foci of fat necrosis were present about the pancreas.

The relationship of this change in the peripancreatic fat to nonsuppurative panniculitis is not clear as the two lesions had very dissimilar histological compositions. Since the patient was a chronic alcoholic, the peripancreatic fat necrosis may have been on this basis. Fatty change in the liver was present in this case as well as in the case reported by Kritzler.[1]

It was also possible to study the various lesions from their apparent incipiency to their more advanced and apparently healed stages. The findings were in agreement with those in the case reported by Shaffer [3] in which the earliest lesion had been described as consisting of small collections of lipid-containing macrophages. The lesion in our case was soon followed by nonsuppurative necrosis which became more extensive and infiltrated with lymphocytes, polymorphonuclear leukocytes and an occasional multinucleated giant cell. The lesion finally showed fibrosis (Figs 1 to 4).

Questions concerning the etiology and the relationship to the co-existing conditions present in this case must remain unanswered. There was no history of bromide or iodine ingestion as has been reported in some of the cases. Sulfathiazole was given after the appearance of these nodules and, therefore, could not bear any causal relationship. The areas of purpura, associated with uremia in this case, were for the most part unrelated in position to the characteristic nodules.

Summary

1. A second case of Weber-Christian's disease with necropsy is presented.

2. Characteristic lesions were present in the mesenteric, pretracheal and omental fat, in addition to those in the subcutaneous adipose tissue.

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[*Illustrations follow*]

DESCRIPTION OF PLATE

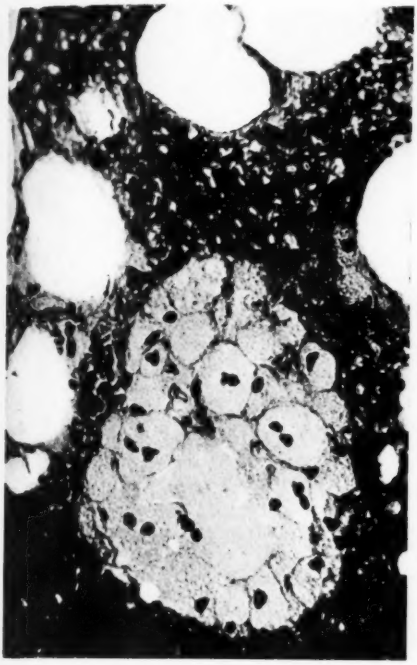
PLATE 147

FIG. 1. Section through an early lesion showing a collection of fat-laden macrophages. Trichrome stain. × 320.

FIG. 2. Section through a nodule in the pretracheal fat showing a small necrotic area. Hematoxylin and eosin stain. × 40.

FIG. 3. Section of the nodule in the mesenteric fat showing fat-laden macrophages. Hematoxylin and eosin stain. × 320.

FIG. 4. Section through an older nodule showing fibrosis. Hematoxylin and eosin stain. × 130.



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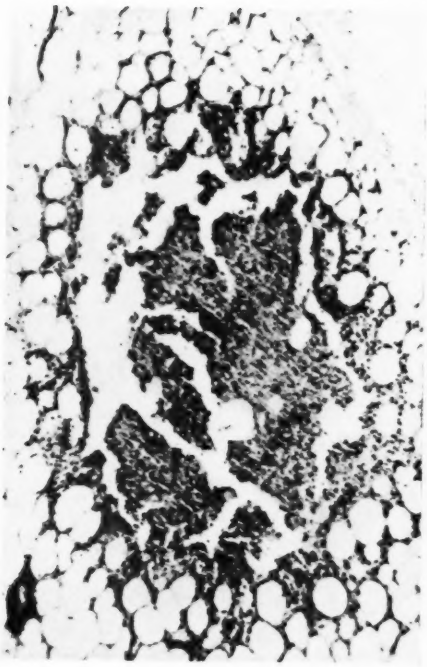

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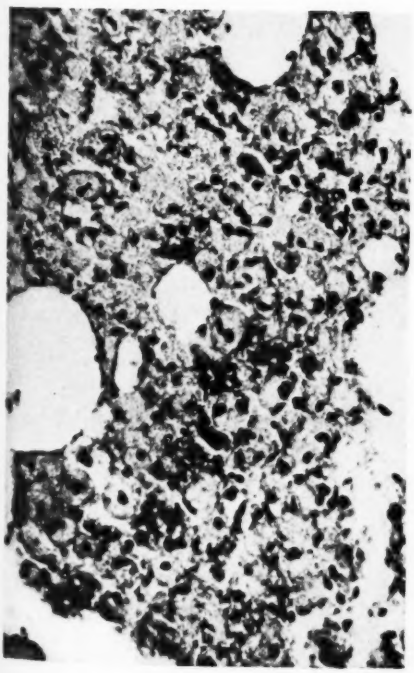

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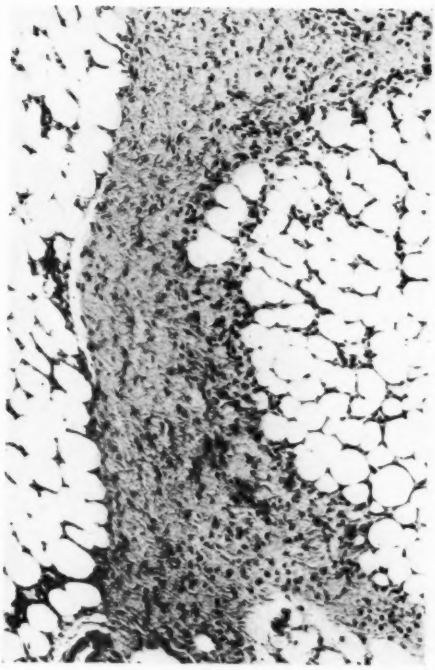

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Spain and Foley

Nonsuppurative Nodular Panniculitis

MULTIPLE PRIMARY LIPOSARCOMAS*

LAUREN V. ACKERMAN, M.D.
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Adipose tissue should be considered as an organ subject to its own diseases. Wells[1] stated it very well: "Adipose tissue is not merely a common connective tissue loaded with stored simple fats but, to a large extent, structurally, developmentally and functionally an independent special tissue more after the order of the true ductless glands." We see systemic diseases such as von Recklinghausen's neurofibromatosis, lymphosarcoma, plasma cell myeloma, and others fairly frequently. It seems reasonable, therefore, that fat tissue, behaving as a single organ, could be involved by a malignant process.

True liposarcomas are certainly rare,[2] and the occurrence of multiple primary liposarcomas is practically unknown in this country. The European literature, however, offers four articles on this subject which will be reviewed later.[3-6] Liposarcomas can occur in any area in which fat is normally found, but arise most frequently in the popliteal spaces, the areas about the buttocks, and in the retroperitoneal areas. They often appear encapsulated, but it is probable that this encapsulation is false, as recurrences after surgery are common.

The following case demonstrates the appearance of multiple primary malignant fatty tumors which were located in the adipose tissue, grew with varying speeds, recurred in some areas, and finally caused death after 10 years.

REPORT OF CASE

F. M. (E. F. S. C. H. no. 3281), a white male, 54 years old, was admitted to the hospital on September 18, 1941. In 1933, a lump in the right inferior gluteal fold had been noted, and in 1938 it was removed at the Bell Memorial Hospital in Kansas City. It shelled out easily. One and one-half years before admission to this hospital, the patient fell, striking his left hip, and 6 months later a hard, painless swelling above the left hip bone was felt. This grew slowly. For 1 year prior to admission, there had been pain on the outer surface of the left thigh radiating down to the foot. Four months previous to admission, he noticed a lump in the right popliteal space. Weakness of the legs had been marked for 2 weeks. Physical examination revealed a well developed white male who was quite weak, preferring to lie in bed. On examination of the abdomen, there was a large, smooth, firm mass felt in the right lower quadrant just beneath the iliac crest. In the left lower quadrant, there was a similar but smaller mass. In the soft tissues of the back, just above the iliac crest on the left, there was a smooth, round, elevated tumor mass measuring about 20 cm. in diameter and elevated about 6 cm. above the surrounding skin (Fig. 1). It was not ulcerated or attached to the overlying skin. There were also several small nevi and one *café-au-lait* spot. The extremities showed signs of wasting and the tendon reflexes were absent. These changes were thought

* Received for publication, September 29, 1943.

to be due to pressure on nerves by the large abdominal masses. Just beneath the right popliteal fossa in the soft tissues, there was a smooth, round, movable mass measuring about 8 cm. in diameter. Roentgenograms following a barium enema showed displacement of the cecum, proximal ascending colon and terminal ileum by an extrinsic, homogeneous tumefaction of the right lower quadrant.

On October 3, 1941, the tumor in the popliteal space was excised; on October 31st the mass on the back was resected; on December 6th the abdominal mass was incompletely resected. The patient was discharged from the hospital on December 24th. He was readmitted on February 14, 1942, because of recurrence of the tumor in the retroperitoneal space. This was given intensive x-ray therapy totaling 7600 r. over four large fields, but the tumor regressed only slightly. He was discharged from the hospital on April 7th. He returned on June 6th, much worse. In the right supraclavicular fossa, there was a hard, movable mass about 5 cm. in diameter, which was removed. After this, he returned to a county home, gradually lost strength, and died on November 23, 1942.

All of the surgically excised tumors had many similar characteristics. They varied in size from 75 gm. (popliteal space) to 1200 gm. (retroperitoneal space). They were all in the soft tissue and shelled out easily. Their external surfaces were lobulated, and showed numerous convolutions. In the grooves formed by these convolutions ran a fine network of small blood vessels. On section, they cut with varying degrees of resistance depending on the amount of connective tissue present. The first one removed cut the easiest and looked very much like brain tissue. Its appearance conformed to Shaw's[7] description of liposarcoma. All of the specimens showed varying degrees of hemorrhage and necrosis relative to the cellularity and vascular supply.

Autopsy

Anatomic diagnoses. Multiple primary liposarcomas, arising from the subepicardial, mediastinal, mesenteric, peritoneal, retroperitoneal, subcutaneous, and bone marrow fat; metastatic liposarcoma of the liver.

The body was emaciated and showed numerous well healed surgical scars. There was a residual, soft tissue tumor of the right thigh measuring about 10 by 8 by 4 cm., and another in the soft tissue of the right popliteal space. At the initial incision, tumor was found in the second and third costal interspaces, apparently prolongations of a tumor arising from the mediastinum. In the peritoneal cavity, the small intestines were adherent to the lower half of the abdominal wall. The liver was rotated counter-clockwise about 90 degrees, so that the normal superior aspect was lying on the right side of the body. As a whole, the liver was lying in the left upper quadrant, displaced and rotated by a large mass of neoplastic tissue occupying the normal liver space. On the surface of the liver were several sharply delineated, yellowish white nodules measuring from 1 to 6 cm. in diameter. On

section they were soft, circular and projected slightly above the cut surface. Tumor completely filled the right iliac fossa and right lumbar gutter and was covered by the iliopsoas muscle. In the left lower quadrant, a similar mass of firm, grayish yellow neoplastic tissue incompletely filled the left iliac fossa. Lymph nodes were apparently replaced by tumor and measured up to 2 cm. in diameter. The right pleural cavity contained a large mass of soft, friable neoplastic tissue which filled one-half to two-thirds of the space, compressed the lung, pushed its way through the intercostal spaces, and caused deviation of the mediastinum to the left. The left pleural cavity was free from tumor. The heart weighed 300 gm. and its subepicardial fat was markedly reduced in amount. At the apex, questionably arising from the subepicardial fat, there was an elevated tumor nodule measuring 3 cm. in diameter which had invaded the myocardium. The right lung showed marked compression by tumor, but there was no definite tumor within the lung parenchyma. The left lung was normal. The other organs showed no gross evidence of tumor.

Microscopic Examination. The fat from the pleura, pericardium, mesentery, peritoneum, popliteal space and mediastinum showed numerous tumor nodules. The cells varied considerably in size and shape, had pale blue vesicular nuclei, and fine nucleoli. In many instances, the nuclei had been compressed to a crescentic outline by the large amounts of fat present. Other tumor cells showed oval, homogeneous nuclei with clear, pale cytoplasm and well defined cellular outlines. Sudan III stain showed large amounts of fat which was almost entirely cytoplasmic. Best's carmine stain for glycogen demonstrated large amounts of cytoplasmic glycogen within practically all tumor cells. In the cells where globules of fat had compressed the cytoplasm, this compressed area was stained positively for glycogen. The tumor in the liver was clearly demarcated, had an apparent connective tissue capsule, and was similar to tumor observed elsewhere. The tumor thought to represent lymph node replacement was shown to be merely nodules of tumor of the same size as lymph nodes. Connective tissue stroma was extremely variable, and numerous areas of necrosis were observed. Thrombosis of the small vessels was widespread.

Comment

This patient had numerous fatty, soft-tissue tumors arising in areas where fat is normally present. Tumor was found in the three most common sites: the popliteal spaces, buttocks and retroperitoneal spaces, as well as other and rarer locations. There was recurrence of tumor growth after excision in both the popliteal space and the thigh.

This type of liposarcoma grows rather slowly. There are numerous examples of solitary liposarcoma reported in the literature with short or no follow-up histories. It is possible that some of those patients may eventually develop other tumors.

There are four cases in the foreign literature which illustrate the occurrence of multiple primary fatty tumors. In 1925, Fahr and Lubarsch[3] described a case entitled "A Metastasizing Lipoma" in which there were multiple tumors, apparently of fatty origin, present around the kidney, in the retroperitoneal region, in the mesentery, serosa, posterior mediastinum, the apex of the heart, the liver and the bone marrow. The authors thought that the original tumor arose from the fat around the kidney and metastasized to the other sites. Nienhuis,[4] too, reported a case of multiple fatty tumors involving mesentery, retroperitoneal tissues, pancreas, extradural tissues and thoracic vertebrae. These tumors were of at least 2 years' duration and Nienhuis concluded that only the vertebral and extradural lesions were metastatic, the others representing primary malignant fatty tumors. He stated that the case reported by Fahr and Lubarsch presented the same picture, rather than a single primary liposarcoma with metastases. In 1934, Siegmund[5] reported a beautiful example of multiple primary malignant fatty tumors occurring in a male of 65 in whom the first tumor was noted in the soft tissue of the thigh. At autopsy, there were numerous individual fatty tumors arising within the pleural cavity, omentum, mesentery, serosa of small and large intestine, along the aorta, surrounding both kidneys, in the soft tissues of the back, thigh and upper arms, the subepicardial fat, and in the bone marrow. He concluded that this was a systemic malignant disease of fatty tissue and should be designated as a "lipoblastische Sarkomatose." He emphasized that a parallelism might be drawn between this and other systemic diseases such as neurofibromatosis and lymphosarcoma. He also independently came to the conclusion that Fahr and Lubarsch's case represented multiple primary tumors. Goormaghtigh,[6] in his extensive review of malignant fatty tumors in 1936, commented on Siegmund's case and reported an additional case of a 59-year-old woman whose first tumor was located in the left half of the abdomen. Radiotherapy was of no value. At autopsy, numerous individual malignant fatty tumors located in the soft tissue of the thigh, the base of the heart, in the gastrocolic, gastrosplenic and suspensory ligament of the liver, were found. There were numerous other tumors arising from the fat of the serosa of the small and large intestine, omentum and mesentery, making a total of 219 individual tumor masses. There was

no evidence of lymph node involvement. The bones, unfortunately, were not examined.

These four well documented cases and the case here reported represent multiple primary malignant fatty tumors and give further support to the conception that adipose tissue should be considered as a single organ subject to its own diseases.

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[*Illustrations follow*]

DESCRIPTION OF PLATES

PLATE 148

FIG. 1. Profile view of soft-tissue liposarcoma of the back.

FIG. 2. External surface of retroperitoneal tumor, showing lobulations.

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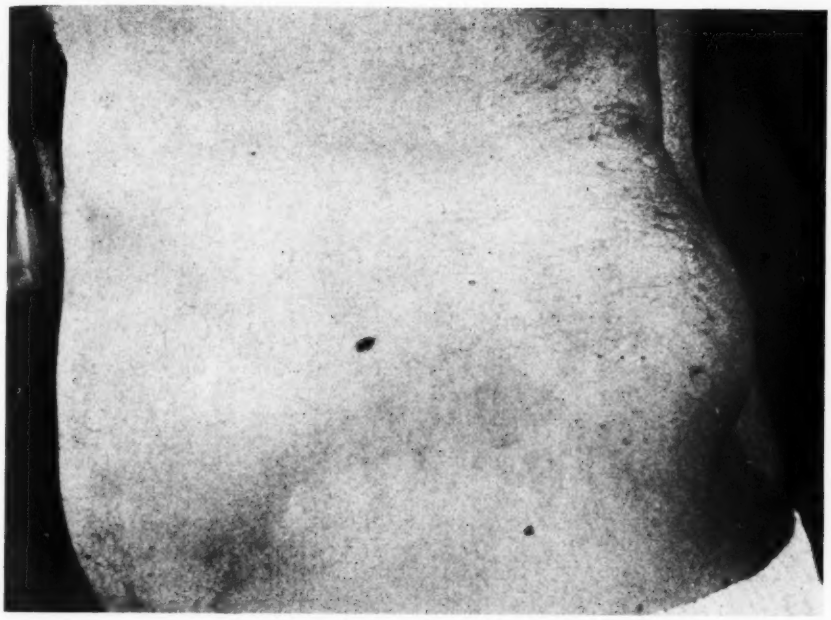

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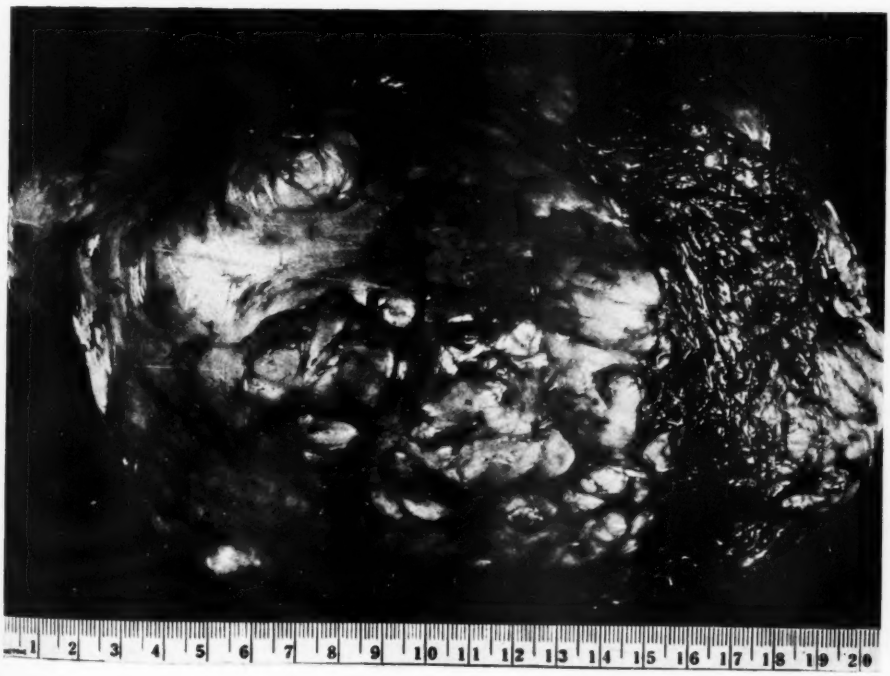

Ackerman

Multiple Primary Liposarcomas

PLATE 149

FIG. 3. Cut section of retroperitoneal tumor.

FIG. 4. Characteristic structure of one of the soft-tissue liposarcomas. × 303.

FIG. 5. Tumor replacing liver parenchyma. × 97.

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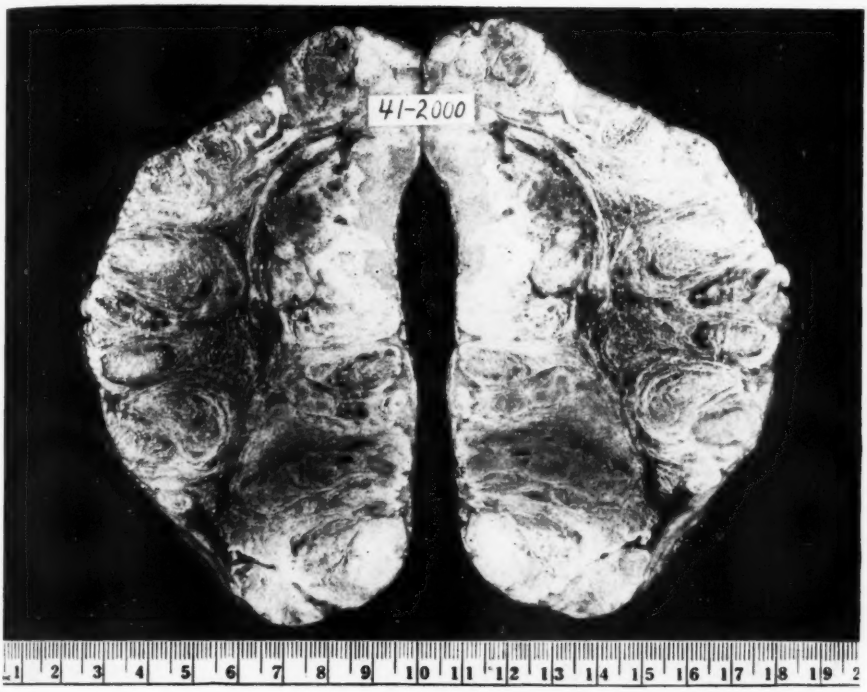

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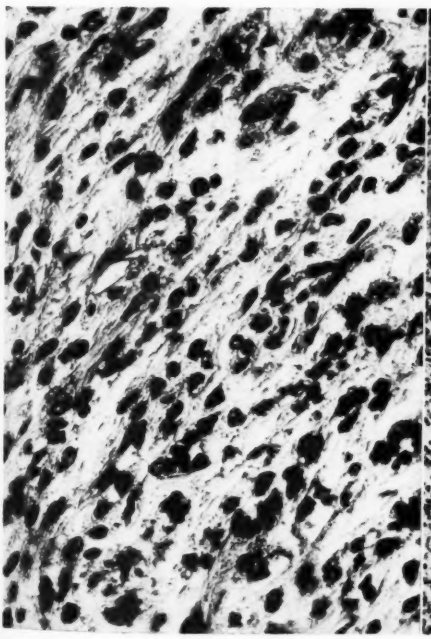

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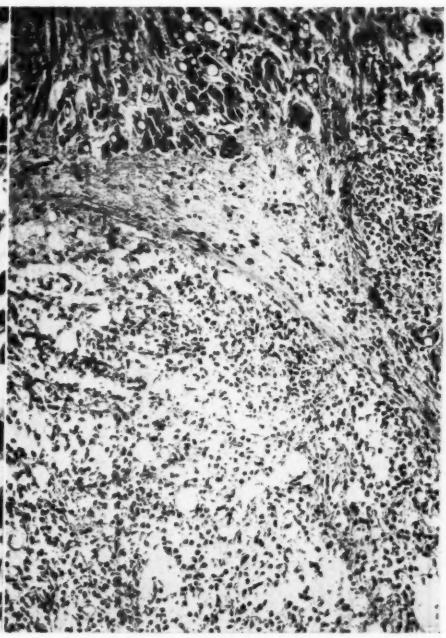

TRUE HERMAPHRODITISM

Report of a Case with Mammary Carcinoma *

John D. Moriarty, M.D.†
(From the New Hampshire State Hospital, Concord, N.H.)

Because of the psychologic and sociologic implications of hermaphroditism, as well as its obvious biologic significance, the problems raised by this condition merit thoughtful consideration. *True* hermaphroditism, as illustrated in the case herein presented, may be defined as the exceedingly rare condition in which both testis and ovary are found in the same individual, either separately or in combination as an ovotestis. According to a careful study by Young,[1] only 20 confirmed cases had been reported in the world's literature before 1937.

False hermaphroditism is a relatively common condition which may closely simulate the true type. The distinguishing feature of pseudohermaphroditism is the presence of gonads of only one sex, whatever combinations of accessory sex organs and anomalies of external genitalia may be present. Over 2,000 cases of false hermaphroditism have been described. Careful investigation is required to differentiate the two conditions, and the diagnosis of true hermaphroditism cannot be confirmed without either biopsy or autopsy studies. As Novak[2] pointed out, many patients with the "feminine type" of hermaphroditism are really females in whom a tumor of testicular type, such as an arrhenoblastoma, has developed in the ovarian substance. The resulting structure is, of course, entirely different from an ovotestis but may give rise to the same manifestations.

To the following case, none of these objections can apply. The pathologic studies are extensive and conclusive and are supported by the clinical data.

Report of Case

Clinical Findings. The patient was a former shoe-worker, married and 79 years of age. For the preceding 8 or 9 years he had shown gradual deterioration of personality and intellect, becoming querulous, irritable and confused. The patient had always been feminine in appearance and reactions, but at 26 years of age he had married a maternal type of woman, with whom he made an apparently satisfactory adjustment despite his total inability to perform coitus. Every month until middle life he "menstruated," with severe physical pain accompanying the process. Family history was irrelevant except for gastric cancer in the mother, cancer (unspecified) in one sibling, and psychosis (unspecified) in another sibling.

Physical examination upon admission disclosed little except for very small external genitalia of the male type, with no right testis palpable, and feminine secondary sex characteristics. The penis was only 1½ inches in length. The scrotum was

* Received for publication, October 4, 1943.
† Now in the Medical Corps, U. S. N.

well formed. There was no evidence of hypospadias, nor of any vaginal opening. Laboratory data were noncontributory.

In the hospital the patient was depressed, agitated and whining. He wandered about aimlessly and showed marked clouding of the sensorium and loss of intellective resources. Three weeks after admission there developed thrombosis of a femoral vein, and subsequently he died of pulmonary embolism.

Psychiatric Diagnosis. Senile psychosis, depressed and agitated type.

Macroscopic Findings

The pertinent findings have been abstracted from the report of the autopsy.

Pelvic Organs. The bladder was of normal size and appearance. However, on the posterior surface there was a firm ridge, running diagonally from the left side of the base of the bladder upward and to the right. This was about 12 cm. long and varied from 1½ to 2 cm. in diameter. On section through this, numerous small cystic cavities were seen, located approximately in the center of firm muscular tissue, which formed the bulk of this ridge. This represented an anomalous and distorted uterus. From the apex of this organ there extended a small, thin, cord-like structure which passed over the pelvic brim and terminated near a firm, white, wrinkled structure measuring 2 by 1½ by ½ cm. The latter resembled in every respect a senile ovary, both externally and on section.

In the left half of the scrotum there was a small, firm, fibrous, brownish red testis, with a fair-sized but atrophic-appearing epididymis. The spermatic cord on that side was quite well developed, and the vas deferens could be identified. In the right side of the scrotum no evidence of testis or epididymis was found. The prostate was in its usual location at the neck of the bladder. It was of approximately normal size and consistency and on section appeared normal.

Breasts. Both breasts were enlarged but not pendulous. The nipples were small and of the male type. The left breast contained a firm white mass (4 by 4 by 2 cm.), which was fairly circumscribed but whose edges were not sharply defined. The tissue had the appearance of a carcinoma. The glandular tissue of both breasts had considerable fatty tissue incorporated in it, but itself appeared abundant.

Brain. The brain was slightly smaller than normal. The arteries at the base showed only moderate arteriosclerosis. The ventricles were somewhat enlarged because of the cerebral atrophy. The pituitary body appeared slightly larger than usual but was otherwise normal.

Gross Pathologic Diagnoses. Pulmonary embolism with infarction; moderate cerebral arteriosclerosis, with minimal generalized arteriosclerosis; cardiac dilatation; true hermaphroditism, with the presence of a prostate, one testis, one ovary and a uterus; feminine type of

secondary sex characteristics and enlarged breasts; cerebral atrophy (senile?); carcinoma of left breast.

Microscopic Findings

Ovotestis. The sections of ovotestis showed two distinct types of tissue arrangement. Approximately one-third of the area consisted of densely packed, elongated nuclei in a dense network of hyaline fibers surrounding typical corpora albicantia (Figs. 1 and 2). The other part of the section was composed of finely fibrillar, scantily nucleated, fibrous tissue; this contained clumps of large cells with spherical and oval nuclei and clearly demarcated, abundant eosinophilic cytoplasm (Figs. 3 and 4). The structure of these clumps was identical with that of the islets seen in the atrophied testicle in the scrotum. Adjacent to the border of this portion of the section were irregular tubular structures lined by low-columnar, nonciliated epithelium.

Testis. Tubular structures of the testis were simulated by finely reticular connective tissue, packed together in areas defined by compact, densely staining, sparsely scattered connective tissue nuclei. The areas thus outlined were of the size and shape of atrophied testicular tubules. In addition there were clumps of large cells with spherical and oval nuclei and with eosinophilic, clearly demarcated cytoplasm (Figs. 5 and 6).

Prostate. The prostate showed irregular glands with tall columnar epithelium in a fibromuscular stroma. Many glands contained corpora amylacea.

Uterus. The section of uterus appeared as the cross section of a tube with smooth muscle and little connective tissue. The lumen was lined by columnar cells, in places arranged as tubular glands surrounded by stromal cells, like those of atrophied endometrium.

Breast. There were fibrous bands in the breast containing ducts, singly and in groups, with abundant fat between the bands. Another section showed acini adjacent to ducts. Some of the acini contained homogenous eosinophilic material.

Breast Nodule. The breast nodule consisted of strands and nests of anaplastic epithelial cells, some forming atypical glandular structures invading atrophied breast tissue (Figs. 7 and 8).

Pituitary Gland. The varying types of cells were distributed in normal proportion and all lobes appeared normal.

Microscopic Pathologic Diagnoses. Atrophied testicle and epididymis; atrophied ovotestis; atrophied uterus; adenocarcinoma of breast; female type of atrophied breast (gynecomastia); infarcts of lung; embolism of pulmonary artery; bronchopneumonia; infarcts of kidney; lipoid lymphocytosis of spleen (type not determined).

Tabulation of Clinicopathologic Features
(as Suggested by Young [1]*)*

1.	Source of material	autopsy
2.	Age	79
3.	Reared as	male
4.	Emotions	male(?)
5.	Bodily aspects	male external genitalia; feminine secondary sex characteristics
6.	Menses	from puberty to middle life
7.	Phallus	not hypospadiac
8.	Vaginal opening	none
9.	Uterus located	along posterior surface of bladder
10.	Right gonad	true ovotestis
11.	Left gonad	testis in scrotum

Comment

Although this patient's deficiencies were hazily appreciated early in life, he led outwardly a fairly conventional male existence. In contrast to some hermaphrodites, who boast of their sexual capabilities, he passively accepted his situation. Libido remained at an infantile level; potency was nil. From a psychosomatic standpoint, it is very interesting to note that when he succumbed to the senium, his psychosis took the form of the depressed and agitated type, indicating his fundamental sense of insecurity.

A noteworthy finding was the carcinoma of the breast. The possible endocrine factors underlying this development constitute a stimulating subject for speculation.

In the earlier investigation of hermaphroditism, the anatomic aberrations were described in great detail, with little or no attention being paid to physiologic and psychologic features. More recently, with the stressing of the functional aspects, the endocrine side of the picture has been illuminated. It is now well known that the urine of even a normal person contains hormones of both sexes. Thus in the urine of a normal male the ratio of excretion is usually about 5 androgenic units to 1 estrogenic unit, and vice versa in a female. It is not surprising, therefore, to find in the psychologic make-up of every man some feminine characteristics. In other words, the difference between man and woman is in many ways more apparent than real. When viewed in this light, a problem such as homosexuality becomes more understandable. Recent endocrine studies tend to support the hypothesis that there is a disturbance in the balance of sex hormones in this condition. For instance, Glass, Deuel and Wright [3] found that sex

hormone assays in a group of male homosexuals yielded androgen:estrogen ratios which appeared to be significantly lower than those obtained in a group of normal males. Thus the majority of patients labeled "homosexual" might more properly be described as "bisexual" and may be regarded as "psychologic hermaphrodites."

Although it is beyond the scope of this paper to discuss the treatment of hermaphroditism, it might be well to mention that in younger patients the outlook is frequently far from hopeless. For instance, Doss and Priestley[4] described good results in a 12-year-old child, treated by surgical removal of all female tissues, preservation of all male tissues, and postoperative administration of testosterone. Here, again, success was furthered by treatment of the total individual rather than of an anatomic condition. Psychosomatic features and endocrine interrelationships must be carefully considered.

Summary

In an aged patient with senile psychosis, true hermaphroditism was confirmed by the demonstration of a right ovotestis and left scrotal testis. Secondary sexual characteristics were of feminine type. In the left breast there was a primary adenocarcinoma. The occurrence of this neoplasm invites attention to the endocrine features of hermaphroditism, which, together with the psychologic aspects, far outweigh the anatomic state in importance. Practical application of the data obtained from the study of hermaphroditism may be utilized in promoting a better understanding of such widespread clinical problems as that of homosexuality.

I wish to express my appreciation to Dr. David Dial (deceased) of the New Hampshire State Hospital and to Dr. R. E. Miller of Dartmouth Medical School for the pathologic reports; and to Dr. W. W. Greulich of Western Reserve University Medical School for the photomicrographs.

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[*Illustrations follow*]

DESCRIPTION OF PLATES

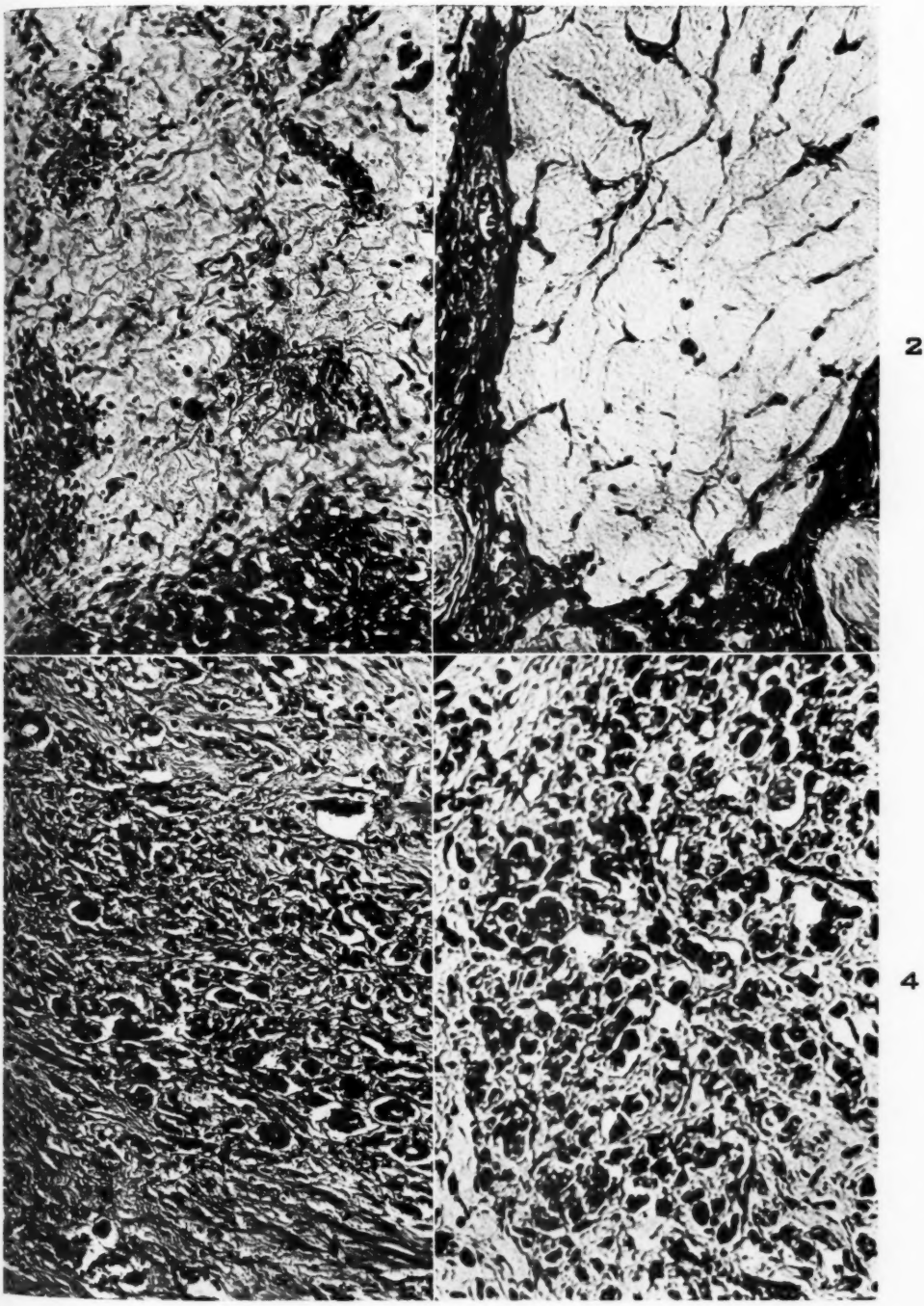
PLATE 150

FIG. 1. Section of ovotestis, showing corpora albicantia. × 129.

FIG. 2. Section of ovotestis, showing corpus albicans. × 247.

FIG. 3. Section of ovotestis, showing clumps of interstitial cells. × 129.

FIG. 4. Section of ovotestis, showing clump of cells of testicular type. × 247.



Moriarty True Hermaphroditism

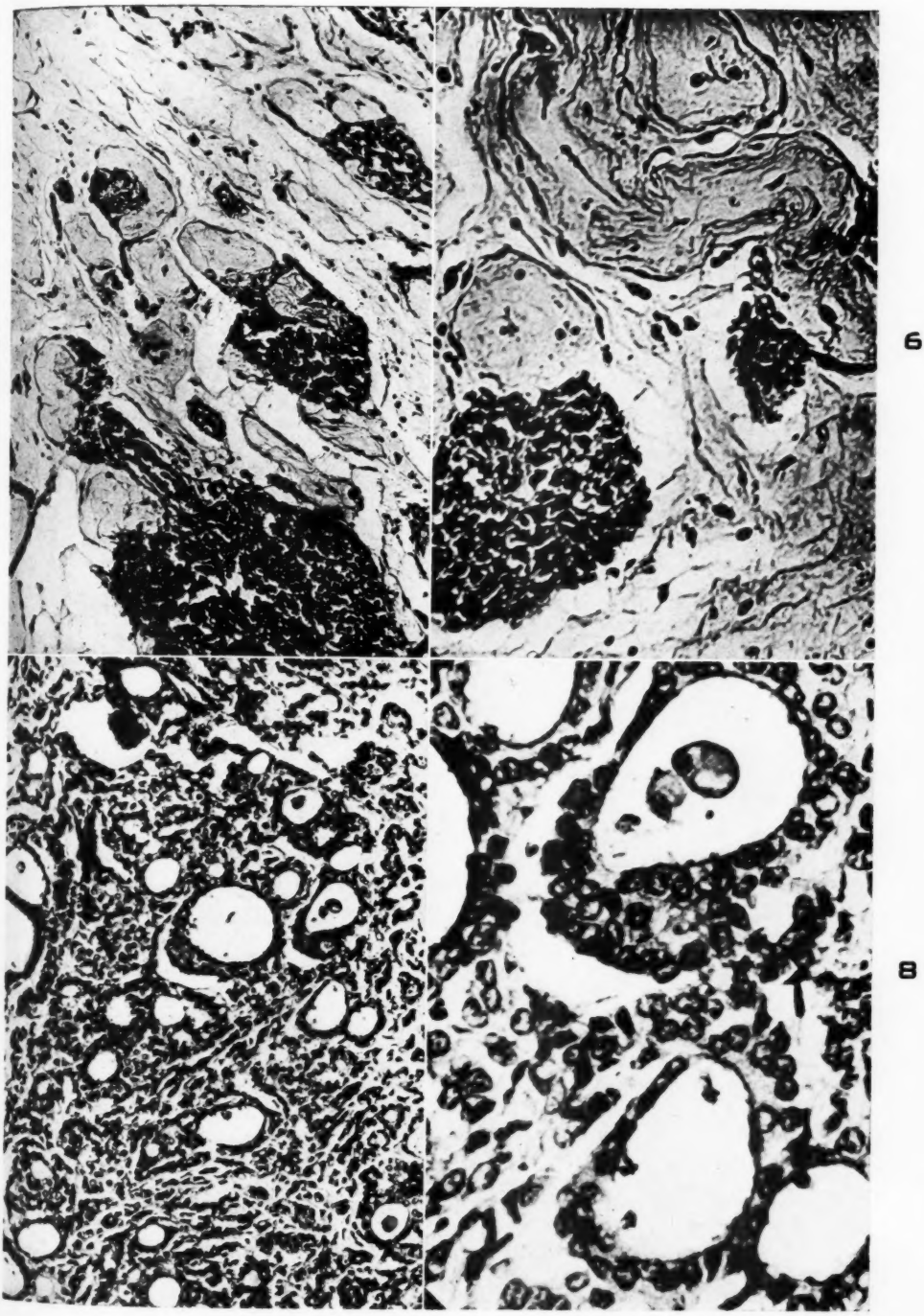
PLATE 151

FIG. 5. Testis showing "ghosts" of tubules and groups of interstitial cell clumps. × 129.

FIG. 6. Testis with atrophic, fibrotic tubules, and interstitial cells. × 247.

FIG. 7. Adenocarcinoma of breast. × 129.

FIG. 8. Detail of structure of the mammary adenocarcinoma. × 505.



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8

Moriarty

True Hermaphroditism

THE EFFECTS OF 3,4 BENZPYRENE ON WOUND REPAIR IN THE SKIN OF MICE *

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The regenerative power of the epidermis differs in various species and, under the same experimental conditions, depends upon the number of cells present in the original epithelial layer.[1] In the rat and pigeon, a skin defect heals more slowly than a defect of equal size in the guinea-pig.[2] The epidermis of the guinea-pig has, in a given area, more cells than the skin of the rat and pigeon. The more numerous the epidermal cells, the greater is the speed with which skin defects are repaired.[2-4]

Accordingly, it might be assumed that within the same species, hyperplastic skin may show greater regenerative power than normal skin. Since an early effect of carcinogenic hydrocarbons on the skin is hyperplasia of the epithelium, a study of the regenerative processes taking place in skin thus rendered hyperplastic was thought a suitable test of this assumption. It might at the same time give some further insight into the mode of action of these carcinogenic substances on the epithelial cells.

MATERIAL AND METHODS

Sixty-six male and female mice of the closely inbred strains, C57 black and leaden, 6 to 8 weeks old, were used. The animals were kept on a diet of Purina Chow and water, both being available at all times.

The mice were divided into three groups. The experimental group consisted of 33 animals receiving applications of 0.3 per cent 3,4 benzpyrene dissolved in benzene. The hair over the lower back was carefully clipped without injury to the underlying skin; 8 drops of the benzpyrene solution were applied at each treatment by means of a dropper. The treatment was repeated three times weekly for periods of 2 weeks and 1, 2 and 3 months, respectively. After each of these periods, a circular piece of skin and subcutaneous tissue, measuring 3 mm. in diameter, was excised from the painted area with a pair of curved scissors. Regeneration was allowed to take place for 3, 5, 8 and 10 days.

Two groups of animals served as controls: one, of 17 mice, was treated with the solvent benzene alone; the second control group, con-

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sisting of 16 animals, was untreated. In both control groups, excisions were made similar to those in the benzpyrene group, and equal periods of time were allowed for regeneration.

In 16 of the mice receiving benzpyrene dissolved in benzene and in all of the animals receiving benzene alone, a second piece of skin was removed from an untreated area of the skin as additional control material.

At the end of the experimental periods, the animals were killed with chloroform. In view of the diurnal mitotic rhythm present in the skin of mice,[5-7] the animals were sacrificed at the same time of the day, namely, between 10 A.M. and 12 noon. A piece of epidermis comprising the area of the excision, together with the adjoining skin, was removed, stretched on filter paper, fixed in 8 per cent solution of Formalin (3.2 per cent formaldehyde), embedded in paraffin, sectioned, and stained with hematoxylin and eosin.

The changes taking place (1) in the marginal epithelium surrounding the defect, and (2) in the new epithelium that grows out from the periphery towards the center of the wound will be described separately. The outgrowing epithelium formed a tongue with a broad insertion and a more or less pointed tip.

The number of rows of cells in the marginal as well as in the regenerating epithelium was determined. In the outgrowing epithelial tongue, the cell rows at its insertion and in the advancing tip were counted separately. The length of the advancing tip was measured with an object micrometer. Our method follows closely that used by Loeb and his co-workers [1-4] in their studies on wound repair.

Mitoses were counted in each of the areas specified. Since mitoses are much more frequent in the hair follicles than in the rest of the skin, only mitoses in the epidermis between the hair follicles were counted. The number of mitoses under the various experimental conditions is given in multiples of the normal. This seemed preferable to giving absolute figures because it allows for the variations existing in various areas.

OBSERVATIONS

A. IN NORMAL MICE

At all stages throughout the 10 days of observation subsequent to excision, the wound was covered with a scab that steadily decreased in size.

Histological Examination

(1) Marginal Epithelium. The epidermis consisted of 2 rows of cells. The upper layer was composed of flat, slightly keratinizing elongated spinous cells; the deeper layer consisted of cuboidal basal cells. The ratio between basal and spinous cells was 2:1 to 3:1.

Mitoses were found only in the basal cells at the ratio of 1:850 basal cells, or 1:1200 epithelial cells including the spinous cells.

At 3 and 5 days following excision, the epithelial layer was thickened and markedly keratinized. The basal cells were 30 to 50 per cent larger than usual; they assumed a cylindrical shape and were oriented in a direction perpendicular to the surface. Their nuclei stained more lightly with hematoxylin than ordinarily; the chromatin had the shape of thin, elongated threads, and the nucleoli had disappeared. These changes were most accentuated in areas close to the margin of the wound. Mitotic proliferation of the basal cells was increased and reached a maximum of from four to six times the normal rate.

Eight and 10 days after operation, there was a gradual return to the resting stage. The cells again became smaller, the nuclei stained more deeply with hematoxylin, and the nucleoli were more definite. The number of mitoses had declined, but was still two or three times greater than usual.

(2) New Epithelium. Hypertrophic epidermal cells grew out from the margin of the wound in a tongue-like manner under the blood clot that covered the defect of the skin. At its insertion, this tongue consisted of at least 3, but more frequently of 4 or 5 rows of hypertrophic cells. At its tip, the tongue was thinned out and was composed of 1 to 3 rows of cells. The farther the cells had advanced into the wound, the more they were stretched out.

Mitotic proliferation was at from four to six times the normal rate at its peak, which was found 5 and 8 days after excision. The movement of the cells was most active during the first 5 days. At later stages, cell migration was slowed down. In one of four examples, the defect began to close 10 days after operation. Details of these changes are given in Table I.

B. IN MICE TREATED WITH BENZENE

The skin underwent thickening and increased keratinization. After 2 to 3 months of treatment, there was some loss of hair in some animals. As compared with the untreated mice, wound repair was hastened. Eight days after operation, the wounds were covered with a small crust. After 10 days, the site of excision was difficult to recognize, whereas in the untreated animals there was still a small defect covered with a scab at this stage.

Histological Examination

(a) Treatment for 2 Weeks and (b) for 1 Month

(1) Marginal Epithelium. The epidermis consisted of 2 or 3 rows of cells instead of 2 as in untreated mice. In the latter, pieces of skin

350 μ long contained from 58 to 68 cells (Table IV, column 4), the ratio between basal and spinous cells being 2:1 to 3:1 (Table IV, column 6). In the benzene-treated animals, on the other hand, pieces of epidermis of the same length contained 78 and 86 cells respectively, and the ratio of basal and spinous cells had shifted in favor of the spinous cells (1:1 and 1.3:1 instead of 2:1 to 3:1). There was increased keratinization in the hair follicles and in the surface epithelium at some distance from the wound. Scattered polymorphonuclear and mononuclear leukocytes were seen in the subcutis. During the first 5 days fol-

TABLE I

Normal Animals

Days after excision	No. of animal	No. of rows of cells in			Length of tongue (*mm.*)	No. of mitoses in multiples of the normal	
		Marginal epithelium	Insertion of tongue	Tip of tongue		Marginal epithelium	New epithelium
3	40	2	3–4	2	0.28	2–3	0
	68	2	2–3	1–2	0.18	2–3	0
	67	2	4–5	2–3	0.31	4–6	2–3
	49	2	3–4	2	0.29	4–6	2–3
5	35	2	4–5	2–3	0.38	4–6	4–6
	73	2	4–5	3	0.42	4–6	4–6
	12	2	4–5	2–3	0.33	4–6	4–6
	48	2	5	3	0.46	4–6	4–6
8	22	2	4–5	2–3	0.52	2–3	4–6
	69	2	4–5	2–3	0.62	2–3	4–6
	47	2	4	1–2	0.58	2–3	4–6
	46	2	5	3	0.67	2–3	4–6
10	31	2	4–5	3	0.72	2–3	2–3
	74	2	4	2–3	0.69	2–3	2–3
	45	2	4–5	2–3	0.78	2–3	2–3
	27	2	4–5	3	Closing	2–3	2–3

lowing excision, the epithelial cells were about 50 per cent larger than ordinarily, and had undergone marked proliferation. Mitotic division began sooner after operation and was more intensified than in untreated controls (Table II). Whereas in the latter, mitoses had increased four to six times at 5 days after excision, in the benzene-treated mice the corresponding values were six to nine times the normal. Eight and 10 days after operation, the number of mitoses had declined and had, in some instances, returned to normal in contrast to the untreated mice in which the number of mitoses dropped more slowly.

(2) New Epithelium. At its insertion, the tongue revealed 4 to 8 rows of cells (3 to 5 in untreated mice). The maximum thickness was reached 5 days after excision. The cells grew out more rapidly towards the center of the wound than in the untreated animals. In the latter, 3 days after operation, the tongue was 0.27 mm. long (maximum, 0.31

mm.; minimum, 0.18 mm.); in the benzene-treated animals, 0.33 mm. (maximum, 0.35 mm.; minimum, 0.31 mm.). After 5 days, the corresponding figures were, for the untreated animals, 0.40 mm. (maximum, 0.46 mm.; minimum, 0.33 mm.); for the benzene-treated mice, 0.57 mm. (maximum, 0.60 mm.; minimum, 0.53 mm.). After 8 days, the tongue was 0.60 mm. (maximum, 0.67 mm.; minimum, 0.52 mm.) long in the untreated animals; in the two benzene-treated mice, the tips of the tongues advancing from both sides of the excision had met, indicating the beginning closure of the defect, whereas only in one of

TABLE II

Animals Treated with Benzene

Duration of treatment	No. of days after excision	No. of animal	No. of rows of cells in			Length of tongue (mm.)	No. of mitoses in multiples of the normal	
			Marginal epithelium	Insertion of tongue	Tip of tongue		Marginal epithelium	New epithelium
2 weeks	3	41	3	5	3	0.35	4–6	4–6
	5	36	2–3	6–7	3–4	0.53	6–9	4–6
	8	39	2	5–6	2	Closing	2–3	2–3
	10	32	1–2	6	2–3	Closing	1	2–3
1 month	3	75	2–3	4–5	2–3	0.31	4–6	2–3
	5	51	2–3	7–8	3–4	0.60	6–9	6–9
	8	76	2–3	5–6	2–3	Closing	1	4–6
	10	65	2	6–7	3	Closing	2–3	2–3
2 months	3	4	2–3	4	2–3	0.29	2–3	2–3
	5	13	2–3	7–8	3–4	0.61	6–9	6–9
	8	59	3	6	2–3	Closing	1	2–3
	10	23	2–3	7–8	3	Closing	2–3	2–3
3 months	3	52	3	5–6	3–4	0.38	4–6	4–6
	5	53	3	9	4	0.69	6–9	6–9
	8	3	3	6–7	3–4	Closing	2–3	2–3
	8	5	2–3	4–5	2–3	0.62	2–3	4–6
	10	28	2–3	7	3	Closing	1	2–3

the four untreated mice had the wound begun to close 10 days after operation (Tables I and II).

The tip of the tongue consisted of 2 or 3 rows of cells after 3 days, and of 3 or 4 rows 5 days after operation; in the later stages of regeneration there was a slight decrease in the thickness of the tongue of cells.

The mitotic count was increased over that seen in untreated controls and reached its peak (six to nine times the normal) 5 days after excision (Table II); after 8 and 10 days, when the wounds began to close, the number of mitoses dropped sharply.

(c) Treatment for 2 and (d) for 3 Months

(1) Marginal Epithelium. After 2 and 3 months there was no further intensification of the growth processes nor of keratinization as compared with conditions seen after 1 month of treatment. However,

some degenerated epithelial cells and cell débris were now found in the cutis. Moreover, the hypertrophy of the regenerating epithelium receded more slowly than in the mice treated for 1 month only. Ten days after operation, the cells had not returned to the resting state.

(2) New Epithelium. The outgrowing tongue was somewhat thicker at its insertion (4 to 9 rows of cells) than in the animals treated for 1 month (4 to 8 rows of cells); neither was there much difference in the length and thickness of the outgrowing tongue of cells (Table II).

C. IN MICE TREATED WITH BENZPYRENE

After application of benzpyrene for 2 weeks, depilation of the skin was accentuated in most animals. The epidermis was rough and brittle, markedly thickened and covered with a thick layer of keratin. These changes increased under prolonged treatment. Of the 33 mice, 4 had papillomas; in 1 of these a carcinoma had developed. In 1 animal a carcinoma was seen without previous appearance of papillomas. Details are given in Table III.

As was the case in the benzene-treated mice, wound repair was hastened after 2 weeks and, in some instances, after 1 month of application of benzpyrene. After 2 and 3 months of treatment with benzpyrene, however, the closure of the wound was, at all stages of regeneration, delayed, and as late as 10 days subsequent to operation the wound defects were still large.

Histological Examination

(a) Treatment for 2 Weeks

(1) Marginal Epithelium. The cytoplasm and the nuclei of the basal cells were larger than after treatment with benzene for a corresponding period. Three days after operation the number of mitoses was increased four to six fold; after 5 days it had reached six to nine times its normal value; after 8 and 10 days, as the wound began to close, mitoses decreased slightly, but their number was still higher than in either untreated or benzene-treated mice of the corresponding stages (Tables I to III). Farther away from the excision, the ratio of basal and spinous cells was 1.3:1 and 1:1 respectively, as had been the case in the benzene-treated animals (Table IV). The epidermis consisted here of 2 to 5 rows of cells as compared with 1 to 3 in the benzene-treated mice. Mitoses had increased to four to nine times the normal number. However, some basal cells exhibited karyorrhexis, karyolysis, or pyknosis. The spinous cells had undergone marked keratinization. The lumina of the enlarged hair follicles were filled with considerable amounts of keratin. Here and there, polymorphonuclear

and mononuclear leukocytes were seen. These changes varied in intensity in different animals.

(2) New Epithelium. The size of the cells and the number of rows

TABLE III

Animals Treated with Benzpyrene

Duration of treatment	No. of days after excision	No. of animal	No. of rows of cells in			Length of tongue (*mm.*)	No. of mitoses in multiples of the normal	
			Marginal epithelium	Insertion of tongue	Tip of tongue		Marginal epithelium	New epithelium
2 weeks	3	42	2–3	6–7	3–4	0.39	4–6	4–6
	3	43	3–4	7	4–5	0.44	4–6	4–6
	5	37	2–3	6–7	4–5	0.61	6–9	6–9
	5	38	3–4	8	6–7	0.68	6–9	6–9
	8	24	4	8	2–3	Closing	4–6	6–9
	8	26	3–4	8–9	3	Closing	4–6	4–6
	10	33	3–4	7	2–3	Closing	2–3	4–6
	10	34	3	8	3	Closing	4–6	6–9
1 month	3	77	2–3	5	4	0.29	2–3	2–3
	3	78*	4	6	4	0.28	2–3	2–3
	5	9†	2–3	5	1	0.36	4–6	4–6
	5	50	1–2	6	3	0.54	6–9	6–9
	8	79	2–3	5–6	3	Closing	4–6	4–6
	8	80	2–3	7–8	3	Closing	6–9	6–9
	10	72	3–4	7–8	3–4	Closing	4–6	6–9
	10	81	2–3	5	2	Closing	4–6	4–6
2 months	3	11	4–5	6	6	0.12	6–9	4–6
	3	71	3–4	5	6–7	0.08	6–9	4–6
	5	14	2–3	5	1–2	0.22	6–9	4–6
	5	62	2–3	4–5	1	0.28	10+	6–9
	8	70	2–3	7–8	3	0.26	4–6	6–9
	8	63	3	10–11	2–3	0.31	6–9	10–
	10	25‡	3	11–12	2–3	0.34	6–9	10–
	10	64	2	6–7	1–2	0.28	4–6	10–
3 months	3	66		8	1–2	0.09	6–9	4–6
	3	56		7	2	0.11	10+	6–9
	5	60§		11–12	1	0.17	10+	10+
	5	57¶		9–10	1–2	0.21	10+	10+
	8	61‖		12	2–3	0.22	10+	10+
	8	58		10–11	2	0.18	6–9	10+
	8	6		11–12	1–2	0.33	6–9	10+
	10	29		9–10	1–2	0.29	10+	10+
	10	30		10–11	1–2	0.36	6–9	10+

* Sick, pneumonia found at necropsy.
† Papilloma noted after 50 days.
‡ Papilloma noted after 45 days.
§ Papilloma noted after 52 days.
¶ Papilloma noted after 30 days, changed gradually into carcinoma.
‖ Carcinoma noted after 90 days.

of cells at the insertion of the tongue of cells paralleled the condition in the marginal epithelium. Three days following operation, the cells were much enlarged and the number of cell rows was increased to 6 or 7. The frequency of mitoses was four to six times as great as in normal skin. After 5 and 8 days, the number of rows of cells was 6 to 9; the

mitotic count had further increased. Likewise after 10 days, the number of mitoses remained elevated.

After 3 days, the tip of the tongue of cells was thicker (3 to 5 rows of cells) than in the untreated animals (1 to 3 rows) or in the benzene-treated group (3 rows). The regenerating cells were enlarged and there was intense mitotic proliferation. The nuclei stained lightly with hematoxylin; the chromatin network was fine and delicate. The tips of the tongues of cells measured 0.44 and 0.39 mm. in length, as compared with 0.35 mm. in the benzene-treated and 0.27 mm. in the untreated animals. After 5 days, 4 to 7 rows of cells were counted instead of 2 to 4 as in the benzene-treated and control groups. The cells remained enlarged and mitoses were frequent. The tongues had grown out still further and measured 0.68 and 0.61 mm. respectively as compared with 0.53 mm. in the benzene-treated and 0.40 mm. in the untreated mice. After 8 and 10 days, the wound began to close in all four animals. The cells were still hypertrophic, and mitotic proliferation was elevated over that in both the benzene-treated and control groups (Tables I to III).

TABLE IV

Total Number of Cells, Number of Mitoses in 10,000 Basal Cells and Ratio of Basal to Spinous Cells in a Field of the Marginal Epithelium of the Skin, Measuring 350 μ in Length, 3 Days after Excision

		No. of mouse	Total no. of cells	No. of mitoses in 10,000 basal cells	[Ratio] basal: spinous cells
Normal		40	60	31	2:1
		68	58	27	2:1
		67	68	52	3:1
		49	62	49	2:1
Benzene applied for:	2 weeks	41	78	51	1.3:1
	1 month	75	86	54	1:1
	2 months	4	94	55	1:1
	3 months	52	85	52	1:1
Benzpyrene applied for:	2 weeks	42	70	44	1.3:1
	2 weeks	43	82	51	1:1
	1 month	77	78	53	1:1.2
	1 month	78	86	55	1:1.3
	2 months	11	120	52	2:1
	2 months	71	84	75	1:1
	3 months	66	130	56	2:1
	3 months	56	125	94	2.7:1

(b) Treatment for 1 Month

(1) Marginal Epithelium. Hypertrophic and degenerating cells were frequently found. Three and 5 days after excision, the mitotic activity appeared to be less in three of four instances as compared with the

corresponding stages of wound healing after 2 weeks of treatment. Eight and 10 days after operation, the number of mitoses was the same as after 2 weeks of treatment in two cases. In the other two instances, the mitoses were increased as compared with those found after 2 weeks of treatment. However, the ratio between basal and spinous cells had now changed to 1:1.2 and 1:1.3 respectively (Table IV). Thus, the decrease of mitoses was only relative to the total number of cells, and was not true in proportion to the number of basal cells (Table IV), in which alone mitoses occurred. At some distance from the margin of the wound, the basal cells had not further increased in size as compared with the 2 weeks' stage. Their nuclei had become irregular and were shrunken and condensed. The regressive changes and also keratinization were intensified. The number of cells in a field 350 μ long was the same as after 2 weeks of treatment. The number of rows of cells was 1 to 4; mitoses were now two or three times as frequent as in the normal epidermis (Table III).

(2) New Epithelium. Three days after excision, the tongue at its insertion consisted of 5 or 6 rows of cells. The number of mitoses was two or three times the normal figure. After 5 and 8 days, the mitoses had increased still further (Table III). After 10 days, the cells had decreased in size, but the number of mitoses remained high (Table III).

After 3 days, the tip of the tongue was composed of 4 rows of cells, and the degree of hypertrophy of the cells was similar to that seen after 2 weeks of treatment with benzpyrene. The mitotic count was comparable to the corresponding experiments in the benzene-treated group (Tables II and III). Some nuclei were pyknotic, and the lengths of the tongues were 0.29 and 0.28 mm. as compared with 0.31 mm. in the benzene-treated group and 0.27 mm. in the untreated animals. After 5 days, mitotic proliferation and outgrowth had made progress. The tongues were now 0.36 and 0.54 mm. long as compared with 0.60 mm. in the benzene-treated and 0.40 mm. in the untreated mice. After 8 and 10 days, mitotic proliferation was increased over the values found in the benzene-treated group, and the wound had begun to close in all four cases (Table III).

(c) Treatment for 2 Months

(1) Marginal Epithelium. The mitoses had increased to six to ten times the normal number during the early stages subsequent to excision, and they remained elevated (four to nine times) after 8 and 10 days. Farther away from the wound, the epidermis consisted of 2 to 5 rows of cells; the mitoses here were six to ten times as numerous as ordinarily. Some cells were now twice as large as usual. This increase of size of the

cells resulted in an increase in the thickness of the epithelial layer. Regressive changes and keratinization were even more accentuated than after 1 month of treatment. The cells were closely packed, and there were 100 in a field 350 μ long. The ratio of basal and spinous cells was 2:1 and 1:1 respectively (Table IV).

(2) New Epithelium. The number of rows of cells increased the longer regeneration was allowed to take place. Eight and 10 days after excision, as many as 12 could be counted. The epithelial layer consisted of large basal cells, small cuboidal cells in the midportion and flat keratinizing cells at the surface. The mitoses had increased to more than ten times the normal number, and the increase was the more pronounced the longer the interval after operation. After 3 days, the tips of the tongue of cells were very short, measuring only 0.12 and 0.08 mm. in length, as compared with 0.29 mm. in the benzene-treated and 0.27 mm. in the untreated groups (Tables I to III). While mitotic proliferation was intensified, the newly formed cells, instead of growing out into the wound, piled up on top of one another. Thus, there were 6 or 7 rows of small cells showing downgrowth rather than migration into the defect. Frequently, these cells showed karyorrhexis and pyknosis. After 5 days, the growth of the tongues in length had made slight progress; they had increased to 0.28 and 0.22 mm. respectively, instead of 0.61 mm. in the benzene-treated and 0.40 mm. in the untreated mice. The tongue was very thin and consisted of 1 to 3 rows of cells only. After 8 and 10 days, the outgrowth of the tongue had made very slight progress (Table III). Mitoses as well as degenerative changes in the cells were common. The downgrowth of the cells was accentuated, whereas the lengthwise outgrowth of the cells was inhibited or markedly delayed.

(d) Treatment for 3 Months

(1) Marginal Epithelium. The upward trend of mitotic proliferation that had been observed after 2 months of treatment continued throughout the periods of wound repair. Hyperplasia and hypertrophy of the epithelial cells were marked at some distance from the excision. The epidermis consisted of 3 to 5 rows of cells. In one field 350 μ long, as many as 130 cells were packed together. The ratio of basal and spinous cells shifted in favor of the basal cells (2:1 and 2.7:1; Table IV), due presumably to the mitotic activity which had increased ten times and more above the normal values (Table III).

(2) New Epithelium. Three days after operation, 7 or 8 rows of cells were counted, and after 5, 8 and 10 days, there were 9 to 12. There was, therefore, a notable multiplication of cells, and again the

increase in mitoses became more marked as the interval following excision increased. Large basal and small polyhedral cells took part in the proliferation. However, degenerative changes were common in these cells, and the cells continued to grow downward into the subcutis, whereas their outgrowth into the wound defect was markedly inhibited.

During all stages of wound repair, the outgrowth of the tongue was inhibited to a greater degree than after 2 months of treatment. The tip consisted of 1 to 3 rows of cells only; and while mitotic proliferation was more pronounced than after shorter treatment, karyorrhexis, karyolysis and pyknosis were frequently found. After 3 days, the tongues were 0.11 and 0.09 mm. long, and 10 days after operation had reached only 0.36 and 0.29 mm. respectively. The downgrowth of the small proliferating cells was even more accentuated than it had been after treatment with benzpyrene for 2 months. In this connection it is interesting to note that polymorphonuclear and mononuclear leukocytes had infiltrated the underlying connective tissue and the epidermis, although the intensity of this infiltration varied.

Comment

An analysis of the processes of repair in the skin of normal mice revealed certain relations between the growth processes in the original epithelium and in the new epithelium growing out into the wound defect. The original epithelium reacted to the making of the wound by hypertrophy and hyperplasia that reached a peak after 5 days. The cell migration into the wound was most marked during the first 3 days, whereas the proliferation of the new epithelium had its maximum after 5 and 8 days. Closure of the wound began at the earliest after 10 days and was associated with a decline of the growth processes.

Under the influence of benzene applied previous to excision, wound healing was accelerated with an intensification of both migration and growth of the epidermal cells. These processes reached their peak after 1 month of treatment, after which the rate of wound repair remained fairly constant at this elevated level.

Under the influence of benzpyrene, the course of wound healing depended in some respects on the duration of the treatment given previous to excision. After 2 weeks of application of benzpyrene, wound repair was accelerated over the normal to about the same degree as in the corresponding benzene-treated mice. However, the mitotic count did not recede as rapidly in the benzpyrene-treated mice as it did in the animals treated with benzene. After 1 month of treatment, epithelial regeneration was stimulated less than after 2 weeks of treatment. After 2 and,

still more so, after 3 months of application of benzpyrene, there was a definite inhibition or retardation of epithelial migration into the wound, as compared with the normal, while epithelial proliferation on the contrary was still further increased. This intensified mitotic proliferation at the margin of the wound caused both a piling up of the cells and a downgrowth into the underlying tissue.

The repair in the skin of normal mice resembles that described by Loeb and his co-workers [1-4] in rats, pigeons and guinea-pigs. There is a cycle of cellular proliferation and mitotic activity, and the process of healing is most active at a time when the wound is largest.

Benzene accelerates the growth processes and the curve of wound repair and does it increasingly, until a certain maximum has been reached beyond which there is no further stimulation. Such a maximum is seen not only at the site of wound healing itself, but also in the skin at a distance from the area of excision.

A growth-stimulating effect of benzene on the epidermis has recently been reported also by Rous,[8] who observed the formation of papillomas in rabbits painted with benzene for long periods of time.

After 2 weeks and 1 month of application of benzpyrene, the proliferation of the epidermal cells and their movement into the wound defect were stimulated. This stimulation was similar to that seen after treatment with benzene alone; it might, therefore, be attributable partly to the action of the benzene used as a solvent for the benzpyrene. But if the excisions were made after 2 and 3 months of application of benzpyrene, the migration of the cells was inhibited, whereas their proliferation was further increased.

As to the cause of these changes in the reaction of the epidermal cells, the prolonged application of the carcinogenic agent may have resulted in injury to the epithelial cells that becomes manifest as a loss of motility. The occurrence of leukocytes may indicate such a toxic action of benzpyrene. In addition, changes in the base of the wound might interfere with cell migration. Since this form of movement represents a stereotropic reaction,[9] it cannot take place without the presence of a solid base that furnishes a support for the migrating cells. In mice treated with various carcinogenic agents including benzpyrene, Orr [10] observed transformation of the collagen of the dermis into a fine-fibered type and also alterations in the texture of the elastic tissues of the cutis. Such changes at the wound base might not only inhibit the migration of the epidermal cells but might also facilitate the downgrowth of the proliferating epithelium into the underlying tissue. However, further investigations will have to test the correctness of this assumption.

In the nonexcised epidermis, we noted a slow, gradual stimulation of the epithelium, when mitoses were counted in a given number of basal cells. The temporary depression of mitotic proliferation found by Champy and Vasiliu [11] in tarred mice and by Cooper and Reller [12] in mice painted with methylcholanthrene may be due to their method of counting.

The normal skin of mice shows not only a diurnal rhythm of proliferation, but also a constant transformation of mitotically proliferating basal cells into spinous cells that no longer undergo mitotic division. The spinous cells have lost their proliferative power, probably because they are removed further from the subcutis, their source of nourishment. In the normal epidermis of the mouse, the ratio of basal and spinous cells is 2:1 to 3:1. In the course of 2 months of treatment with benzpyrene, and incidentally with benzene also, this ratio changes to 1:1.3 in favor of the nonproliferating cells of the surface of the skin (Table IV). If the latter cells are excluded from the count, the mitotic index in the basal cells is found to increase steadily under the influence of benzpyrene.

SUMMARY

In the skin of normal mice, wound repair takes place primarily by proliferation and hypertrophy of the epidermis adjoining the wound and by movement of the epithelium into the defect. These processes are hastened by the application of benzene to the skin previous to the making of the wound. Under the influence of benzpyrene applied for 2 weeks previous to excision, both components of wound healing are likewise accelerated. After prolonged treatment with benzpyrene, proliferation of the epidermal cells is further intensified, whereas cell migration into the defect is inhibited or almost suspended.

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